A STUDY ON THE BACTERIOLOGICAL PROFILE AND ROLE OF BIOFILM FORMING ORGANISMS IN CATHETER ASSOCIATED URINARY TRACT INFECTIONS IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

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for the award of the degree of

M.D. (MICROBIOLOGY)
BRANCH – IV

CHENGALPATTU MEDICAL COLLEGE,
THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI – TAMILNADU
MAY 2018

CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON THE BACTERIOLOGICAL PROFILE AND ROLE OF BIOFILM FORMING ORGANISMS IN CATHETER ASSOCIATED URINARY TRACT INFECTIONS IN A TERTIARY CARE HOSPITAL''** is a bonafide record of work done by **DR.I.GUNAPARVATHY,** during the period of her Post graduate study from 2015 to 2018 under guidance and supervision in the Department of Microbiology, Chengalpattu Medical College and Hospital, Chengalpattu – 603 301 in partial fulfilment of the requirement for **M.D. MICROBIOLOGY** degree Examination of The Tamil Nadu Dr. M.G.R. Medical University to be held in MAY 2018.

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DECLARATION

I declare that the dissertation entitled **"A STUDY ON THE BACTERIOLOGICAL PROFILE AND ROLE OF BIOFILM FORMING ORGANISMS IN CATHETER ASSOCIATED URINARY TRACT INFECTIONS IN A TERTIARY CARE HOSPITAL"** submitted by me for the degree of M.D. is the record work carried out by me during the period of **March 2016 to February 2017** under the guidance of Professor **Dr. A.VIJAYALAKSHMI, M.D., M.B.A.,** Department of Microbiology, Chengalpattu Medical College, Chengalpattu. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfilment of the University regulations for the award of degree of M.D. Microbiology (Branch IV) examinations to be held in May 2018.

Place: Chengalpattu
Date

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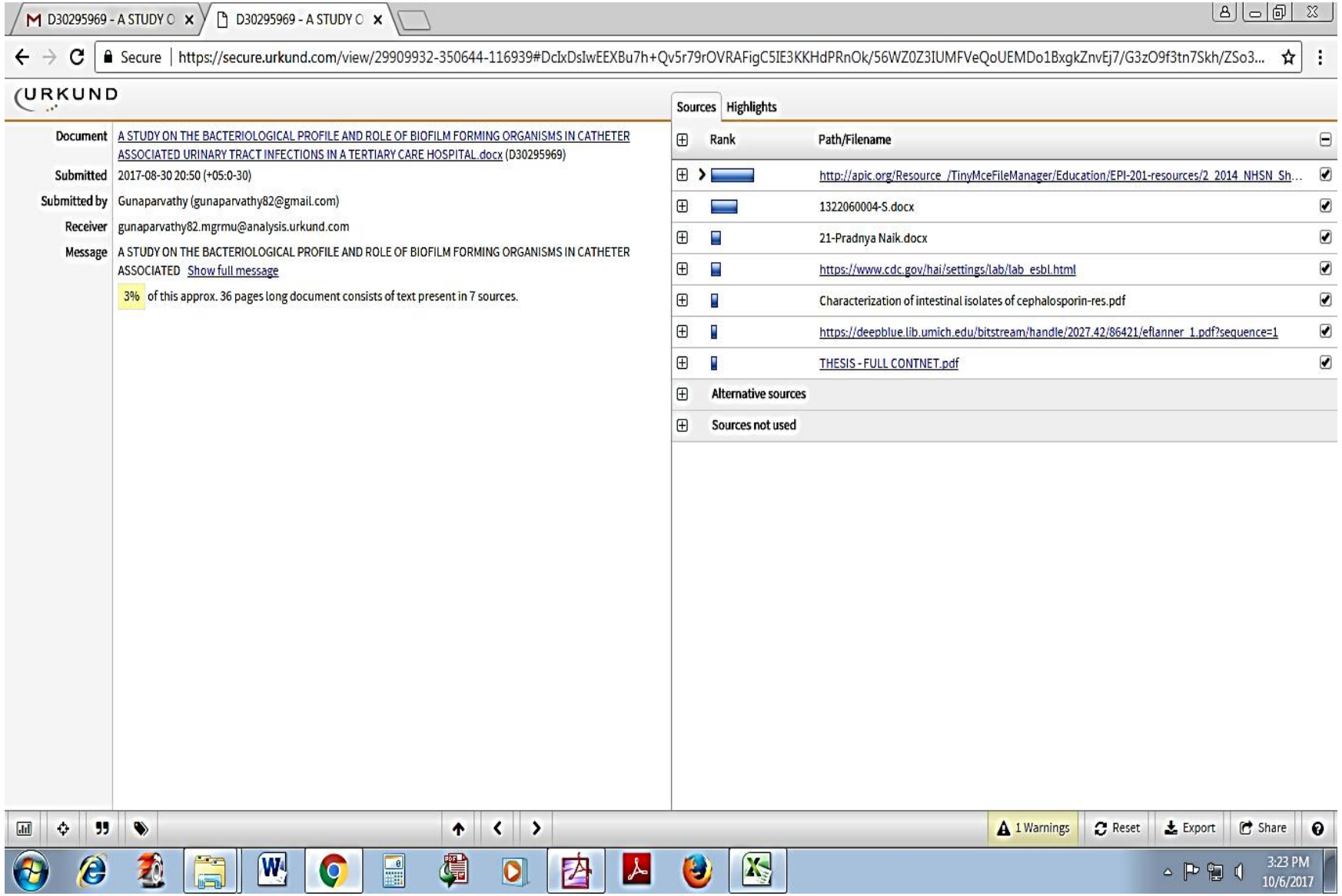
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ABSTRACT

A STUDY ON THE BACTERIOLOGICAL PROFILE AND ROLE OF BIOFILM FORMING ORGANISMS IN CATHETER ASSOCIATED URINARY TRACT INFECTIONS IN A TERTIARY CARE HOSPITAL

BACKGROUND :

Catheter associated urinary tract infection (CAUTI) is a major cause of illness and poor response to the treatment is due to biofilm formation by the organisms, which are usually multi drug-resistant.

AIM :

To estimate the prevalence and identify the organisms causing CAUTI and to identify biofilm producing properties of organism by three phenotypic methods and their resistant pattern.

Materials and Methods:

This Cross sectional study was conducted in Chengalpattu Medical College and Hospital for one year (March 2016 to February 2017). 309 urine samples from catheterized patients in the Department of Urology were collected and processed in Department of Microbiology by standard protocol. Biofilm formation was detected by Congo Red Method (CRA), Tube Method(TM) and Tissue Culture plate Method (TCP).

Results:

Significant bacteriuria was observed among 207/309 (67%) samples. Gram negative bacteria (GNB) were predominant isolates 162(78.2%) and among GNB, *Escherichia coli* was commonly isolated 81(39.13%) followed by *Klebsiella spp* 36(17.39%), *Proteus spp*. 22(10.63), *Pseudomonas aeruginosa* 22(10.63%) and *Citrobacter koseri* 01(0.48%).They were mostly sensitive to imepenam (100%), followed by piperacillin-tazobactam (97%). Among 207, 33(15.94%) were Gram positive cocci. The most common in GPC were *Staphylococcus aureus* 16(7.73%), followed by *CoNS* 10(4.83%) and *Enterococcus spp*. 07(3.38%). They show higher sensitivity to Vancomycin (100%). Prevalence of Biofilm producers detected by TCP were 122(63%), TM 100 (51%) and CRA 42(22%). Antimicrobial resistance among the biofilm producers were Methicillin resistance organisms 12(92%), ESBL producing organisms 49(78%) and AmpC β-lactamase producers 9(60%).

CONCLUSION:

Detection of biofilms can be recommended for recurrent infection before institution of empirical antibiotics. TCP is a good screening method for detection of biofilm in resource limited settings.

Keywords : CAUTI, Biofilm, ESBL, Amp C β-lactamase, TCP.

INTRODUCTION

INTRODUCTION

Catheter associated urinary tract infection (CAUTI) is a major clinical problem which leads to considerable morbidity and mortality (15%).[1] The overall prevalence of CAUTI accounts for up to 40% of all the nosocomial infections in which 80% of them contribute to UTI.[2] The major cause of the illness and the poor response to the treatment is due to the biofilm formation by the organisms, which are usually multi drug-resistant. The predominant form of life for many of the microbes in any hydrated system is by the formation of a cooperative adhesion matrix termed as “Biofilm”.

An organized biofilm includes adherence of the microorganisms either to a surface or to each other by quorum sensing, a change in gene expression resulting in a different phenotype from the planktonic state and an extracellular matrix that is composed of host components and secreted by the bacterial products. If a urinary tract infection occurs, the inherent defense mechanism encounters and protects the individual. Whereas in the catheter surfaces, the inherent defense system is masked which are due to hydrophobic and electrostatic interactions and with the help of flagella, the organisms move and attach to them. Then it is followed by cell division, recruitment of other bacteria and secretion of extracellular matrix. The exchange of nutritious material occurs by cell-to-cell signaling.

The major problem with biofilm formation by the microorganisms is drug resistance and resistance to defense mechanisms. This is achieved by simple shear forces which are resistant to phagocytosis and resistant to antimicrobial agents, which adds up the survival advantage for the microbes. Among the nosocomial UTI, *Escherichia coli* is the most the common organism,[3] followed by *Klebsiella spp.* and then *Pseudomonas aeruginosa*. The development of bacteriuria is mainly attributed to the duration of catheterization in the inpatient that in turn increased the length of hospital stay, cost and morbidity.[4] So it is mandatory to know the prevailing bacterial strains and its antimicrobial susceptibility pattern to start an empirical targeted therapy to prevent such sequel. Hence targeted antimicrobial therapy is a must to overcome the CAUTI with biofilms.

Although several studies have been conducted on biofilm detection and methods of detection from CAUTI, limited data is available in our locality about the prevalence of CAUTI. In addition, the different methods and reliability are important to do a systematic analysis of biofilm detection. Molecular analysis of biofilm helps in identification of genes which are most commonly involved and the properties of that particular gene expression. Hence we have planned to conduct a study on the prevalence of CAUTI, its antimicrobial susceptibility pattern, the presence of biofilms and its various methods of detection of biofilms.

AIMS

AND

OBJECTIVES

AIMS AND OBJECTIVES:

- To estimate the prevalence of CAUTI among the study population.
- To isolate, identify and characterize the organisms causing CAUTI.
- To determine the antimicrobial susceptibility pattern of the isolated organisms and to study their resistance pattern in CAUTI.
- To identify the biofilm producing properties of bacterial isolates by Tissue culture plate method, Tube method and Congo red method.
- To compare the different phenotypic methods used for biofilm production.

REVIEW
OF
LITERATURE

REVIEW OF LITERATURE:

Urinary tract infections are the fourth most common type of health care-associated infection worldwide. It accounts for 12% of the infections as reported by the acute care hospitals.[5] Almost most of the health care associated UTI are caused due to the instrumentation of the urinary tract. The term CAUTI has been used for Catheter associated urinary tract infections.

Definition of UTI :[6]

"Urinary tract infections (UTI's) can be defined as bacteriuria (colony count $>10^5$ CFU/ml in adults; $>10^4$ CFU/ml in children) of a urinary pathogen with associated clinical symptoms and signs of urgency, painful micturition, and hematuria. According to the Centers for Disease Control and Prevention (CDC), a symptomatic UTI must meet at least one of the following criteria"

- The patient is not on indwelling urinary catheter at the time of specimen collection or during the onset of signs and symptoms.
- The patient has atleast anyone of the following symptoms or signs with no other recognized causes of fever, urgency, frequency, dysuria, suprapubic tenderness and costovertebral angle tenderness.
- The patient's urine sample shows a positive growth of urinary pathogens of $\geq 10^5$ with no more than 2 species in growth.

Definition of CAUTI[5]:

"UTI, where an indwelling urinary catheter was in place for >2 calendar days of the date of event, with the day of device placement being day 1 and an indwelling urinary catheter was in place on the date of the event or the day before. If an indwelling urinary catheter was in place for > 2 calendar days and then removed, the date of the event for the UTI must be the day of discontinuation or the next day for the UTI to be catheter-associated." (Chart 1)

Chart-1

Urinary tract Infection Criteria

Criteria	Urinary Tract Infection (UTI)
Symptomatic UTI(SUTI)1a Catheter-associated Urinary Tract Infection (CAUTI)	Patient must meet 1,2 and 3 below: 1. The patient had an indwelling urinary catheter that has been in place for >2 days on the date of event (day of device placement =Day 1) and was either: • Present for any portion of the calendar day on the date of event or • Removed the day before the date of event 2. Patient has at least one of the following signs or symptoms: • Fever (>38^{0}C) • Suprapubic tenderness • Costovertebral angle pain or tenderness • Urinary urgency • Urinary frequency • Dysuria 3. Patient has a urine culture with no more than two species of organisms identified, at least one of which is bacterium of ≥ 10^5CFU/ml. All elements of the UTI criterion must occur during the infection window period.

Chart 1: Criteria for the diagnosis of CAUTI (Adapted from CAUTI guidelines 2009 final – Centers for Disease Control and Prevention)

Epidemiology of CAUTI:[6]

An estimated 15-25% of the hospitalized patients will have an indwelling urinary catheter at some point of time during their hospital stay,[7] and the obstruction of the indwelling catheters can lead to sepsis and may even result in mortality.[8] As per the surveillance in the United States, each year around 13,000 deaths is attributed to UTI.[9] The prevalence of CAUTI in health care institutions makes an economic burden, thus implies the necessity for preventing the infection. According to US studies, the cost associated with CAUTI episodes is about 758 dollars per infection and the estimated total cost of treatment and prevention of CAUTI in the United States ranges from 340-450 million dollars annually.[6][10]

Etiology of CAUTI:

Infection is caused by a variety of pathogens including *Escherichia coli, Klebsiella spp., Proteus spp., Staphylococcus aureus, Coagulase negative staphylococcus (*CoNS*), Enterococcus spp., Pseudomonas spp., Enterobacter spp,* and *Candida.* Many of these pathogens are a part of the patients own flora that may act as a source of infection called as endogenous infection. But it can also be acquired by cross-contamination from other patients or hospital personnel or by exposure to contaminated solutions or non-sterile equipments called as exogenous infection.

Pathogenesis of CAUTI:[10]

In spite of innate defense mechanisms against microbial infection of the intact urinary tract, specific organisms are capable of colonizing in this environmental niche. Like other mucosal pathogens, uropathogens also employ specific strategies to infect the urinary tract, which includes colonization of a urinary catheter and/or mucosal site, evasion of host defenses, replication and damage to host cells. The insertion of a foreign body eg: Urinary indwelling catheter into the bladder increases the susceptibility of a patient to UTIs, as these devices serve as the initiation site of infection by introducing opportunistic organisms into the urinary tract.

Among the uropathogens, the majority of them belong to fecal contaminants or skin commensals from the patient's own resident or transient microflora that colonize the periurethral region. Transient floras that originate from hospital personnel or from contact with other patients may represent antibiotic-resistant nosocomial strains thus further more complicating the treatment of these infections. Bacterial entry into the bladder can occur at any time, but most commonly during the time of catheter insertion or through the catheter lumen. The preferred mechanism of bladder entry during CAUTI is extraluminal, which accounts for 66%, where the organisms ascend from the urethral meatus along with the catheter urethral interface. Some organisms can also enter the bladder intraluminally (34%), where the bacteria enter into the bladder as a result of manipulation of the catheter system. The catheter

provides a surface for the attachment of host cell binding receptors that are recognized by bacterial adhesins, thus enhancing microbial adhesion. During the process of insertion, urinary catheters may damage the protective uroepithelial mucosa, which leads to the exposure of new binding sites for bacterial adhesions.[11] Lastly, the presence of the indwelling catheter results in an overdistension of the bladder and incomplete voiding that leaves the increased amount of residual urine for microbial growth.

Gram-negative uropathogens produce an assortment of adhesins including those attached to the tip of the hair-like projections that are known as fimbriae or pili and adhesins that are anchored directly within bacterial cell membranes known as nonfimbrial adhesins. Once firmly attached on the catheter surface, the bacteria change its phenotypic nature and secrete exopolysaccharides that entrap and protect bacteria. Those bacteria that get attached and replicate, form microcolonies that eventually mature into biofilms.

Chart 2:

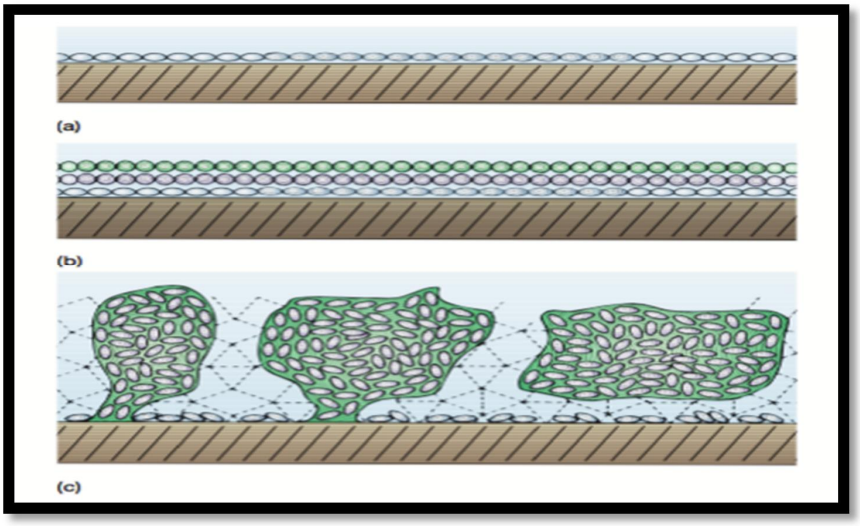


Chart 2: "Biofilms or microbial growths on surfaces as in freshwater and marine environments, can develop and become extremely complex, depending on the energy sources that are available. (**a**) Initial colonization is by a single type of bacterium. (**b**) Development of a more complex biofilm with layered microorganisms of different types. (**c**) A mature biofilm with cell aggregates, interstitial pores and conduits". (Adapted from Microbiology, Lansing M Prescott, 5th edition, page 621)

Definition of biofilm:[6]

"Biofilm can be defined as a microbially derived sessile community consisting of cells that are attached to an interface or to each other, embedded in an extracellular polymeric matrix that is produced by them and demonstrate

an altered phenotype associated with a differential gene expression."[12] This definition also applies to those biofilm cells that have broken off from a biofilm on a colonized medical device like catheters and circulate in the body fluids with the ability to establish itself in another niche.

Biofilm formation and structure:[6]

Biofilms can form on abiotic surfaces such as minerals, air-water interfaces and also on biotic surfaces such as plants, microbes, and other animals. In human body, bacteria reside as residents in the form of biofilms on the skin, oropharynx and nose, intestine and indwelling medical devices. The first step in biofilm formation is the attraction of bacteria to the surface by environmental signals. On reaching the surface, the bacteria then attaches to it as single cells or as clusters. When single cells are attached to a surface, they form a monolayer biofilm. A monolayer biofilm can be defined as one in which the bacteria attach only to the surface.[13] When bacteria are attached to a surface as a cluster, they form a multilayer biofilm. Multilayer biofilms can be defined as a microbial community, where the bacteria are attached both to the surface and also to the neighboring bacterial cells.[13] The type of biofilm formed depends upon the environmental conditions and surfaces that favor their development, the genes that are activated, the architecture of the biofilm and the extracellular matrix composition.[13]

Biofilm formation in indwelling urinary catheters:

Foley catheters are commonly used to manage urinary incontinence in elderly patients and in those with bladder dysfunction. Besides helping the patients, these also put them at high risk for the development of UTIs. Uropathogens such as *Proteus mirabilis, Providencia stuartii, Morganella morganii* are strong urease producers and have a capability of forming a unique type of crystalline biofilms on catheters. The production of urease enables them to breakdown the urea in urine[(14)] and releases ammonia, which raises the urinary pH resulting in calcium and magnesium phosphate crystal formation within the biofilm matrix. Studies have also demonstrated that biofilm is a prerequisite for the crystal formation since the matrix may act as a nucleation site for crystal development.[(15)] Following this, the production of urease by these colonies, calcium and magnesium phosphate crystals begin to form and the biofilm extends down the luminal surface. The crystal formation in-turn leads to the blockage of catheters due to crystallization and encrustation that ultimately ends in bladder distention, urine leakage and pyelonephritis. Additionally, crystalline biofilms can also cause irritation and trauma of the urethral mucosa.[(16)]

Detection of biofilm formation:

Various methods of detection of biofilm formation which includes Tissue Culture Plate method (TCP), Tube method (TM), Congo Red Agar

method (CRM), bioluminescentassa, piezoelectric sensors and examination by fluorescent microscopy. Comparative studies of various methods to detect biofilm formation concluded that for detection of biofilm producing bacteria, TCP can be recommended as a general screening method.

Prevention of biofilm formation:

A systemic review conducted states that out of the proportion of health care-associated infections that can be prevented reveals CAUTI was the most preventable nosocomial infection.[17] An estimate of the number of avoidable cases ranged from 95,483 to 3,87,550 per year and associated lives saved ranged from 2225 to 9031 annually.[6],[17] Prevention of CAUTI is mainly attributed to the criteria for appropriate placement and early removal of catheters. But the recent advances in our understanding of the pathogenesis and key factors in biofilm formation made a good impact in the development of adequate and effective control strategies. Several protective strategies have been suggested for the prevention of CAUTI, some of which are already in place for patient care, whereas others are still in developmental stage. The control strategies include:

- **Limited catheter usage:**[18]

Catheter usage should be selective and limited to reduce the numbers of patients at risk for developing CAUTIs. Like antimicrobial policy, urinary catheters usage should be properly instructed at their need of use, as it has been

overly used in the nosocomial and institutional settings because of their relative ease of application. Several studies suggested that this is the case, as 21 to 38% of initial catheterizations had no justifiable indication.[19] In fact, one survey determined that for 28% of inpatients, the physicians and medical students did not know which of their patients had received catheterization, due to their improper documentation. These types of forgotten catheters could be reminded through the utilization of a computerized reminder system. However, there should be a restriction of urinary catheters to only those patients who strictly require catheterization, such as to relieve urinary tract blockage, to allow drainage of neurogenic bladders and urinary retention, to facilitate healing of the genitourinary tract postoperatively or to measure the urine output in critically ill patients with accuracy. The devices should be promptly removed after their required use to reduce the risk of infection.

The main attributed risk factor is the duration of the catheterization and it was estimated that the risk for developing bacteriuria with catheter insertion is 3 to 10% per day, 10 to 50% up to 7 days and nearly 100% for patients that have long-term catheterization i.e., 28 days.[18] Hence, based on the reviews, it has been recommended that urinary catheters should be changed every 8 to 10 days to reduce the risk of infection. Drainage bags should be emptied at a minimum of every 4-6 hours to prevent the bacteria entering the catheter lumen.

- **Alternate methods of catheterization:**

Many alternative methods of catheterization that potentially reduce the risk of CAUTIs, includes condom and suprapubic catheterization.[18] Condom catheters, while useful for male patients who had bladder outlet obstruction, require meticulous care to avoid complications like skin maceration that may provoke UTI. The use of suprapubic catheters i.e., indwelling catheters inserted above the pubic bone directly into the bladder, shows promise in terms of risk of infection.[20] However, no controlled clinical studies have been conducted to confirm its benefits over the meatal catheterization.

- **Closed system Foley's catheter**:

The introduction of the closed catheter system where one of the collection tubes is fused to the drainage bag is helpful for the reduction of CAUTIs and many variations to this drainage system have been attempted to further decrease the rate of infection. Some of these changes include the addition of a urine sampling port in the drainage tubing and pre-connected collecting tube systems. It was stated that infections associated with indwelling urinary catheters have dramatically reduced from 100% to less than 25% for up to 2 weeks of catheterization due to the usage of closed drainage systems versus the ones that were open.[21]

- **Catheter size:**

When selecting the size of the catheter, smaller catheters (14 French or 16 French) and 5-ml balloons should be utilized,[22] as larger catheters have been shown to be a risk factor for the development of UTI. These larger catheters tend to increase the amount of residual urine that can lead to the reflux of urine into the bladder and subsequently increases the risk of blockage of the periurethral glands that leads to urethral irritation, erosion and infection.

- **Catheters coated with antimicrobial agents:**[18]

The application of antimicrobial solutions and lubricants on the catheter surface prior to catheter insertion and the addition of antimicrobial agents in the collection bag have no proven added benefits as a preventive measure for CAUTI. Conversely few randomized trials state that antimicrobial impregnated catheters containing either nitrofurazone[23] or the combination of the broad - spectrum antibiotics minocycline and rifampin[24] leads to significant reduction in bacterial CAUTIs. However, these trials are limited and the emergence of resistant strains is also not prevented.

Besides antibiotics, impregnation of catheter material with antiseptics, eg: silver compounds, has been a possible preventive measure with conflicting results on the efficacy of these silver-coated urinary catheters. Many Clinical trials have shown promising results as to the efficacy of silver oxide-coated catheters compared with uncoated catheters. The basic mechanism of silver

compounds is that it prevents the adherence of several strains that cause CAUTIs (*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Enterococcus faecalis* and *Klebsiella pneumoniae*) as shown by the radiolabeled-cell assay.

Diagnosis and treatment of CAUTI:[18]

Usually, these infections clear up spontaneously after catheter removal. Also, antibiotic therapy has little benefit for patients with long-term catheterization.[25] This is because bacteriuria occurs very commonly and the treatment of catheterized patients may lead to the emergence and selection of antibiotic resistance and increases the risk for the development of multi drug resistant nosocomial strains. There is a prevailing wrong statement that asymptomatic bacteriuria should not be treated unless the patient is either immunosuppressed or at risk for bacterial endocarditis about to undergo urinary tract instrumentation or pregnant. However, the presence of bacteriuria serves as a reservoir for the potential progression of these organisms into asymptomatic infection. This subsequently leads to significant sequel such pyelonephritis, bacteremia and may even end with sepsis. Therefore, this condition is a major health risk factor for those individuals who are in indwelling urinary catheters.

Virulence factors for *E. coli* [26]

The Fimbria, which binds on urothelium persist within the urinary tract. Three types of fimbriae are Type 'S' fimbriae (S FA-1), Type 'P' fimbriae and Type 'Dr' fimbriae.

Other factors are Siderophores, Toxins-Haemolysin which are subdivided into α-lysine which lyses the RBC and β Lysine which also lyses the RBC's and lymphocytes that inhibit the phagocytosis. Cytotoxic necrotising factor (CNF), 1-2Uropathogenic strain specificproteins (USP), Protectins, TIR Domain-containing proteins (tcp C), Intimin and Colonising factor – CFA I, II, III.

Virulence factors for Klebsiella

Four factors that are virulent in *Klebsiella spp*. are Capsular antigen, Type I and type III pili, serum resistance LPS and siderophore.

Virulence factor for Proteus[27]

Virulence factor of Proteus are Fimbriae or Pilli that facilitate adherence of *Proteus mirabilis* to host tissue such as urinary tract epithelium. LPS or endotoxin causes a series of host inflammatory response which is responsible for Gram negative endotoxin-induced sepsis and urease production by Proteus is an important factor in the pathogenesis of UTI.

Treatment

UTI is commonly treated with cephalosporins, fluoroquinolones and aminoglycosides. Among these third generation cephalosporins are most widely used because of its broad spectrum of activity, low toxicity, ease of administration and its favorable pharmacokinetic profile.[27] Because of their extensive use, they also developed resistance to many organisms especially ESBL producing organism.

Extended Spectrum β-Lactamase (ESBL)

Antibiotic era started with the discovery of penicillin by Alexander Flemming in 1928.[28] Usage of Penicillin started in 1941. The emergence of penicillin resistance was identified in S*taphylococcus aureus* which is due to plasmid-encoded β-lactamase. The first plasmid-mediated β-lactamase in Gram-negative organisms TEM-1 has been described in early 1960's. It is first isolated in *Escherichia coli* from a patient Temoniera in Greece and the gene responsible for it was named after her. It is also spread to other genera soon. Another common plasmid-mediated β-lactamase found in *Klebsiella pneumoniae* and *Escherichia coli* are SHV-1 (Sulphhydryl in Variable). Over the last twenty years many new β - lactam antibiotics have been developed which are resistant to hydrolytic action of β-lactamases but because of their irrational use, these antibiotics also became resistant.

To overcome it, around 1980, third generation cephalosporins which are also called broad spectrum cephalosporins were introduced. Because of their over usage, they also became resistant.

In Germany during 1983, isolates of *Klebsiella pneumoniae* and other Enterobacteriaceae were found to produce a plasmid-determined β-lactamase that hydrolyzed cefotaxime as well as other newer third generation of cephalosporins (eg: ceftazidime, ceftriaxone, cefpodoxime).

This new β-lactamase, called SHV-2 is derived from a mutation in the well-known SHV-1 and it is commonly found in *Klebsiella.*[29] As they lead to resistance for cephalosporins, they are called extended spectrum β-lactamases.[27]

Mechanism of Action of β-lactam antibiotics:[28]

The β-lactam antibiotics act by inhibiting cell wall synthesis of organisms. Bacteria synthesize UDP-N-Acetyl muramic acid penta peptide and UDP-N-Acetyl glucosamine. Once Peptidoglycan residues are linked together, UDP will split off. The final step is cleavage of the terminal D-alanine of the peptide chains by Transpeptidases and the cross linkage between peptide chains of the neighbouring strands is formed. β-lactam antibiotics inhibit trans peptidases so that cross linking is not formed.

Mechanism of bacterial resistance to β-lactam antibiotics:[28]

1. Enzymatic inhibition: It is plasmid-mediated e.g. β-lactamase
2. Membrane impermeability is both plasmid and chromosomal mediated
3. Alteration of target protein e.g. Penicillin-binding protein
4. Enhanced efflux of the drug from the periplasmic space

β- Lactamase:

This is a heterogeneous group of Penicillin recognizing proteins. They are the members of a super family, which are the active site of serine proteases. They act by cleaving an amide bond of beta-lactam ring to form an acyl-enzyme complex. Any β-lactam antibiotic may be inactivated by these enzymes. There are about > 170 enzymes of this kind.[30],[31]

β-lactamase classification[32]

The Early classification scheme has been described by **Richmon** and **Sykes** based on substrate profile and location of genes encoding the β-lactamases. A modern scheme based on the molecular structure has been proposed by **Ambler**. Class A, C and D are serine β-lactamases, where as class B enzymes are metallo β-lactamase that requires zinc for activity.[29]

A Recent classification of β-lactamases is by **Bush-Jacoby-Medeiros** scheme based on substrate profile and inhibition by clavulanic acid.[30]

The broad spectrum plasmid-mediated β -lactamases of Gram-negative bacilli such as TEM-1 and SHV-1 produced by Class A, are stable for many years.

From 1980, a series of enzymatic variants has appeared, that has a broadened spectrum of activity against the newly developed β-lactam antibiotics. These ESBLs are first found in Europe, most commonly in *Klebsiella spp.* less commonly in *Escherichia coli.* The number of enzymes continues to increase.

The new enzymes are located on TEM-1 and SHV-1 plasmids. But they would have been derived originally from a chromosomal enzyme. Many of the new β-lactamases differ from each other only in single amino acid substitution but these changes have profound implications for clinical management of infectious diseases.

Detection of β-lactamases[29]

There are various biochemical tests for detection of β-lactamases enzymes. This is by measuring Penicilloic acid which is produced when β-lactamases hydrolyse benzyl penicillins. The acid production is detected by measuring the change in pH by an indicator dye (**acidimetric method**), by exploiting the ability of penicilloic acid to reduce iodine and reverse the

formation of the blue color when the iodine complexes with starch (**Iodometric Method**) and **Chromogenic Cephalosporin Method** by using Nitrocefin. Nitrocefin is normally yellow but when the β-lactam ring is hydrolyzed it turns red.

β -lactamase inhibitors[32]

These compounds structurally resemble β-lactam antibiotics. They can bind to β-lactam antibiotics either reversibly or irreversibly protecting the antibiotics from their destruction. They serve as **suicide bombers** utilizing all available enzymes. These compounds have weak bactericidal activity but are potent inhibitors of many plasmid-encoded and some chromosome encoded β -lactamases. Three important β-lactamase inhibitors are clavulanic acid, sulbactam and tazobactam.

Clavulanic acid show only lesser antibacterial action but when combined with β-lactam antibiotics, inhibition of bacteria is enhanced which are other wise resistant to β-lactam antibiotics. Sulbactam has broader spectrum of inhibition but less potent, but tazobactam is as potent as clavulanic acid.

Extended spectrum of Beta lactamase

Enzymes capable of hydrolyzing major β-lactam antibiotics including third generation cephalosporins are called as extended-spectrum β-lactamases.

Characteristics of ESBLS:[32]

They are mostly class-A cephalosporinases carried on plasmids. Which are more common in *Klebsiella species* followed by *Escherichia coli* described first in Germany and France.

1) All the enzymes are active against cephalothin.

2) Imipenem and cefoxitin are not hydrolyzed.

3) Comparative activity against cefotaxime and ceftazidime varies with enzymes.

4) Some enzymes active against aztreonam.

5) Inhibition of activity by β-lactamase inhibitors can be demonstrated.

Major risk factors for ESBL production

Risk factors are prolonged stay in ICU, long-term use of antibiotic, nursing home residency, severe illness, higher use of ceftazidime and other Third generation Cephalosporins and use of catheters.

Detection methods of ESBL[32]

There are several methods available to detect the ESBL.

a. Double-disk approximation test of Tarlier

b. Three Dimensional Test

c. Inhibitor Potentiated Disc Diffusion Test

d. MIC Reduction test

e. E- test

f. Phenotypic Confirmation Test

g. Molecular detection methods

a. Double-disk approximation test of Tarlier

The organisms are swabbed onto a Mueller-Hinton agar plate. An antibiotic disk containing one of the Oxyimino β-lactam antibiotics is placed 30mm from the amoxicillin-clavulanic acid disk. The enhancement of zone of inhibition of the Oxyimino β-lactam caused by the synergy of clavulanate present in amoxy-clav disk indicates a positive result.[32]

b. Three Dimensional test

The advantage is simultaneous determination of antibiotic susceptibility and β- lactamase substrate profile. Two types of inoculum are prepared.

a. Inoculum-1: contains $10^9 – 10^{10}$ CFU/ml of active ESBL producers.

b. Inoculum-2: Contains 0.5 McFarland Std. (150 million organisms/ml)

The plate is inoculated as for disc diffusion procedure with inoculum-2. A circular slit of 4mm is cut on the agar and the position at which the antibiotic disks are placed and the colony of 10^9-10^{10} inoculum was poured into it. Distortion in the circular inhibition zone is interpreted as positive for ESBL production.

c. Inhibitor Potentiated Disc Diffusion test

Cephalosporin discs are placed onto MHA plates, with clavulanate and without clavulanate. More than 10mm increase in the zone of inhibition of the clavulanate containing MHA plate indicates ESBL production.

d. MIC Reduction test

An eight fold reduction in the MIC of third generation cephalosporins in the presence of clavulanic acid indicates the production of ESBL.

e. E test:[33]

E test ESBL strips have 2 gradients, on one end ceftazidime and on the opposite end ceftazidime plus clavulanic acid. MIC is the point of intersection of the inhibition ellipse with the E-test strip edge. The ratio of ceftazidime MIC and ceftazidime - clavulanic acid MIC > 8 indicates the presence of ESBLs.

g. Phenotypic Confirmation Test[33]

Antibiotic susceptibility testing is done on Mueller-Hinton Agar plate with 0.5 McFarland's standard of the organism. Lawn culture of the organism is streaked onto MHA and third generation cephalosporin, ceftazidime (30µg) disk is tested alone and along with their combination of clavulanic acid. Organisms with >5mm increase in the zone of inhibition for ceftazidime / clavulanic acid (30µg/10µg) are confirmed as ESBLs. (NCCLS recommends MIC > 2µg/ml for cefotaxime, ceftazidime, aztreonam, ceftriaxone (or) cefpodoxime as potential ESBL producers).[32][29]

Two indicators of ESBLs are

1. Four fold reduction in MIC when the third generation cephalosporins are used with clavulanic acid.

2. 5mm increase in diameter of inhibition zone when using disk diffusion method with third generation cephalosporin and clavulanic acid combined disk.

h. Molecular detection methods:

Tests already described, only presumptively identify the presence of ESBL. Earlier, the determination of iso-electric point is sufficient for studying ESBL, but nowadays since there are >90 TEM type and >25 SHV type of β-lactamase and many of them have same iso-electric point, it has become impossible to detect the individual ESBLs. So the detection of β-lactamases using DNA probes specific for SHV is used but they are labor intensive. The easiest and more reliable molecular method used to detect ESBLs is PCR with oligonucleotide primers that are specific for a β-lactamase gene. Oligonucleotide primers can be chosen from sequence available in Gene Bank. Primers are usually chosen to anneal to regions where various point mutations are known to occur.

Detection of AmpC β-lactamase[34]

a. Disk Antagonism test[35]

The organisms that exhibited resistance to third generation cephalosporins and cefoxitins are swabbed onto a Mueller-Hinton Agar Plate and Cefoxitin (30μg) and Ceftazidime (30μg) disks are placed at a distance of 20mm from center and incubated overnight at 37°C. AmpC β-lactamases inducibility is recognized by blunting of the ceftazidime zone adjacent to cefoxitin disk.

b. Amp C disk test

The test is based on the use of Tris-EDTA to permeabilize a bacterial cell and release β-lactamases onto the external environment. Amp C disks (i.e., filter paper disks containing Tris-EDTA) are prepared in-house by applying a mixture of saline and Tris-EDTA of 20µl in 1:1 ratio to a sterile filter paper disks allowing the disks to dry and storing them at 2- 8°C. The surface of a MHA plate is swabbed with a lawn of cefoxitin susceptible *Escherichia coli* ATCC 25922 according to the standard disk diffusion method. Immediately prior to use, AmpC disks are hydrated with 20 µl of saline and several colonies of each test organism are applied to a disk. A 30µg cefoxitin disk is placed on the inoculated surface of the MHA. The inoculated Amp C disk is then placed almost touching the antibiotic disk with the inoculated side of the disk is in contact with the agar surface. The plate is then inverted and incubated overnight at 35 °C in ambient air.

After incubation, plates are examined for any distortion, indicating significant inactivation of cefoxitin (positive result) or the absence of a distortion, indicating no significant inactivation of cefoxitin (negative result).

c. Modified three-dimensional test

Fresh overnight growth from MHA is transferred to a pre-weighed sterile micro centrifuge tube. The tube is weighed again to determine the weight of bacterial mass to obtain 10-15mg of bacterial wet weight. The

botanical mass is suspended in peptone water and pelleted by centrifugation at 3000 rpm for 15 minutes. Crude enzyme extract is prepared by repeated freeze thawing of the bacterial pellet (approximately 10 cycles). Lawn culture of *Escherichia coli* ATCC 25922 is prepared on MHA plates and cefoxitin 30µg disk is placed onto plates. Linear slits (3cm) are made using a sterile surgical blade, 3mm away from cefoxitin disk. On the other end of the slit, a small circular well is made and the extracted enzyme is loaded. A total of 30-40 ml of the extract is loaded onto the well at 10 µl increment. The plates are kept upright for 5 – 10 minutes until the liquid dries and incubated at 37°C for 24 hours. Enhanced growth of the surface organism at the point where the slit inserted the zone of inhibition of cefoxitin is considered a positive three-dimensional test and interpreted as evidence of AmpC β-lactamase.

Medical significance of detection of ESBL

Patients having infections caused by ESBL- producing organisms are at increased risk of treatment failure with extended spectrum β-lactam antibiotics. So it is recommended that if an organism is confirmed to produce ESBL it is considered as resistant to all third generation cephalosporins.

Treatment

Carbapenems are most effective and reliable as they are highly resistant to the hydrolytic activity of all ESBLs due to the Trans 6-hydroxy ethyl group. Alternatively, fluoroquinolones and aminoglycosides may be used if they show in vitro activity[(36)]. Although clinical data for their use are absent, a β - lactam

and β-lactamase inhibitor combination such as amoxicillin-clavulanate or piperacillin-tazobactam may also be a further option to consider. All these agents should be used with caution, as their susceptibility varies among ESBL producers. Cephamycin, such as cefoxitin and cefotetan, although active in vitro are not recommended for treating such infections, because of the relative ease with which these strains decrease the expression of outer membrane proteins, rendering them resistant. In urinary tract infection combination with clavulanic acid can be used.[36]

Carbapenemases[37]

Klebsiella pneumonia carbapenemases (KPC), although initially identified in *Klebsiella pneumoniae,* has now been recognized in a variety of other Enterobacteriaceae. Organisms possessing these enzymes are often resistant to one or more of the carbapenems.

In vitro susceptibility testing of Enterobacteriaceae containing KPC may indicate that the isolate is susceptible to carbapenems, but therapeutically the carbapenems antimicrobial may not work. Currently no reliable in vitro method exists for identifying these organisms. However, if an isolate possess ESBL and the MIC for carbapenems are 2 or 4 μg/ml, there is a possibility that it may produce KPC-type or some other Carbapenemase. Because these organisms are resistant to carbapenems, infections often are treated with colistin or polymyxin antimicrobials with mixed results.

Confirmatory method for diagnosis of CAUTI:

Bacteriuria can be detected microscopically by Gram staining of uncentrifuged urine specimens, Gram staining of centrifuged specimens or direct observation of the bacteria in urine specimens. Gram stain of uncentrifuged urine specimens is a simple method. A volume of urine is applied to a glass slide, allowed to air dry, stained with Gram stain and examined microscopically. The performance characteristics of the test are not well-defined because different criteria have been used to define a positive test result. The urine Gram stain test has the important advantage of providing immediate information as to the nature of the infecting bacterium or yeast and thereby guiding the physician in selecting an empirical with antimicrobial agents.[38]

Bacteriuria can be detected chemically when the bacteria produce nitrite from nitrate. The biochemical reaction that is detected by the nitrite test is associated with the members of the family Enterobacteriaceae (the pathogens which are most commonly responsible for UTI).

Pyuria can be detected and quantified microscopically by measuring the urinary leukocyte excretion rate, counting leukocytes with a hemocytometer, counting leukocytes in urine specimens using Gram staining or counting leukocytes in a centrifuged specimen. The advantages of urine microscopy are that leukocytes, leukocyte casts and other cellular elements are observed

directly. One disadvantage of urine microscopy is that the leukocytes deteriorate quickly in urine that is not fresh or that has not been adequately preserved. In addition, each of these methods has disadvantages that limit its usefulness as a routine test. Because of these disadvantages, urine microscopy should be limited to patients in whom pyelonephritis or other more serious infections are suspected.

The ideal method is to do quantitative loop method that determines the bacterial number by inoculating the plates using calibrated platinum loops and counting the colonies directly.

Recently, many automated methods of ascertaining bacterial counts are currently available, including staining bacterial cells with safranin (Bac-T-Screen) or acridine orange (Autotrak). Once these screening tests are completed, the presence of CAUTI is confirmed by the determination of bacterial cell counts. Two urine cultures with the repeated isolation of the same uropathogen with 100 CFU per ml of urine or the isolation of a single uropathogen with 10^5 colonies per ml for a patient being treated with antimicrobial agents is indicative of urinary tract infection.

Treatment:

The etiology of CAUTI is dependent upon the patient and illness and the urine culture results are essential to guide the treatment. All evidences support the practice of catheter change during treatment for CAUTI. The goal is to

remove biofilm-associated organisms that serve as a site for reinfection. Pathological studies reveal that many patients with long-term catheters have occult pyelonephritis. A randomized trial in persons with spinal cord injury who were undergoing intermittent catheterization found that, relapse was more common after three days of therapy than after fourteen days. In general, a 7 to 14 days course of antibiotic drugs is recommended.

In the setting of long-term catheter use like 28 days or more, systemic antibiotics, bladder-acidifying agents, antimicrobial bladder washes, topical disinfectants and antimicrobial drainage-bag solutions have been relatively ineffective in preventing the onset of bacteriuria and have been associated with the emergence of resistant strains. The best strategy for the prevention of CAUTI is to avoid insertion of unnecessary catheters and to remove the catheters when they are no longer necessary.

MATERIALS

AND

METHODS

MATERIALS AND METHODS:

Study setting: Department of Microbiology and Department of Urology, Chengalpattu Medical College & Hospital, Chengalpattu

Study Period: One year (March 2016 to February 2017)

Study design: Cross-sectional study

Study population: Patients attending urology OPD and inpatient who fits the inclusion criteria

Sample size: Around 300 (Calculated as per statistical data)

Inclusion Criteria:

- Patients of age > 18 years
- Patients who have been catheterized for more than 48 hours at the time of specimen collection
- Patients who have at least one of the following clinical signs and symptoms like fever (>38°C), costovertebral angle pain and tenderness and turbidity of collected urine.

Exclusion criteria:

- Patients of age < 18 years
- Urinary tract abnormality
- History of antibiotic therapy in last 2 weeks

Ethical consideration:

Approval was obtained from the Institutional ethical committee at Chengalpattu Medical College before commencement of the study. Informed consent was obtained from all the patients participated in this study.

Statistical analysis:

Statistical analyses were carried out using Statistical Package for Social Sciences (SPSS)

Sample collection:

All patients who fulfill the inclusion criteria were included in the study group during the study period. Informed consent was obtained from each patient. The sample collection procedure and implication of study was explained to the patient beforehand. History pertaining to the study was obtained in terms of name, age, sex, length of hospitalization, date of catheterization, previous administration of antibiotics and the presence of any risk factors like diabetes mellitus, obesity, immune suppression.

Collection Of Urine Samples:[39]

Urine sample was collected from catheterized patients. Urinary catheterization helps in collection of bladder urine with less urethral contamination. Specimen collection from such patients was done with strict aseptic precautions. A pair of gloves was worn while handling urinary catheters. The catheter tubing was clamped just above the port to allow collection of freshly voided urine. With 70% alcohol, the catheter port and the wall of the tubing were cleaned vigorously. By using sterile syringe with needle, the urine was aspirated by maintaining the integrity of the closed drainage system to prevent introduction of organisms into the bladder. The collected urine was then transported in a sterile, wide mouthed, screw capped container.

Preliminary examination of urine:[39][33]

- **Physical nature of urine** - odor, color and clarity of urine were first evaluated for the possible presence of urine with a strong odor, hematuria (red urine), pyuria (cloudy urine) or phosphate crystal deposits (cloudy urine).

- **Microscopic analysis of urine** – wet mount in unspun urine sample is examined microscopically for the presence of crystals, urinary casts, epithelial cells, blood cells and bacteria. A count of more than eight pus cells /mm^3[38] is suggestive of pyuria.

Gram staining

A drop of the well mixed uncentrifuged urine was placed on the slide, thin smear was made, air dried, heat fixed and Gram staining was done and examined under oil immersion field. Presence of ≥1 bacteria / OIF was taken as significant bacteriuria which accounts for $>10^5$ CFU/ml and the presence of pus cells was taken as a definite indication of UTI.[39]

Sample processing:

Culture:[40][39]

Before inoculation, urine sample was mixed thoroughly and the calibrated loop was inserted vertically into the urine container and the Urine samples were taken and inoculated onto the Cystine Lactose Electrolyte Deficient Agar (CLED) and Blood agar (BA), starting from the center of the plate the loop was touched and the inoculum was spread in a straight line across the entire diameter of the plate. Without re-entering urine, the loop was drawn across the entire plate in a zigzag manner. The first inoculum was crossed numerous times to produce isolated colonies. (Chart 3) A colony count of $>10^3$ CFU/ml was taken as indicative of a positive culture, as all the urine samples collected were catheterized urine samples (whereas, a colony count of $>10^5$ CFU/ml is significant in case of clean-catch midstream urine).[40]

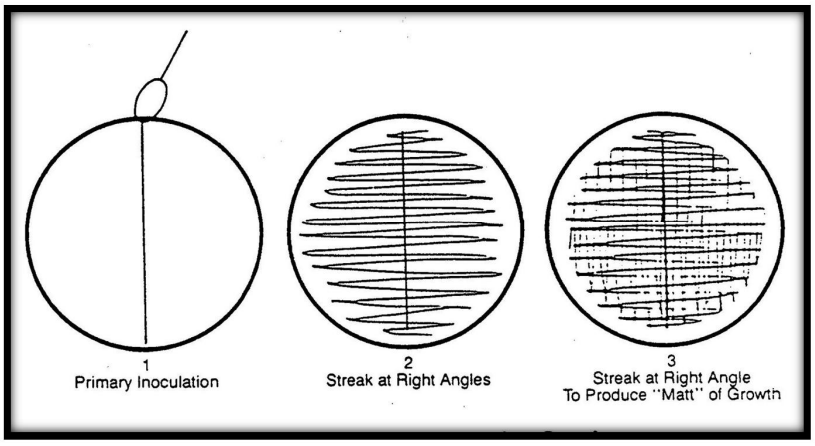


Chart 3 : Method of streaking for semiquantitative bacterial count to account for significant bacteriuria in urine

The inoculated CLED plate was incubated at 37^0C for 24 hours and looked for growth. Those samples that showed positive growth were further processed and biochemical reactions were done to do speciation.

Preliminary tests done for identification of organism were Gram's staining, Catalase test, Oxidase test and Motility testing. After coming to a conclusion of the genus, the specific tests were done to speciate the organism.[(40)]

Chart 4

Biochemical reaction for GPC and GNB

Gram Positive Cocci (GPC)	Gram Negative Bacilli (GNB)
1.Catalase	1.Oxidase
2.Slide coagulase	2.Catalase
3.Tube coagulase	3.Triple sugar iron test
4.Mannitol salt agar	4.Indole test, Methyl Red test, Voges-Proskauer test,Citrate utilization test (IMViC)
5.Urease	5.Mannitol motility medium test
6.Sugar fermentation test	6.Nitrate reduction test
	7.Oxidation-Fermentation tests(OF)
	8.Sugar fermentation test
	9.Moeller's decarboxylase test

Gram positive cocci (GPC):[40]

1) Catalase test:

An isolated colony from the culture to be tested was picked up with a sterile glass rod and inoculated into a test tube containing 3% hydrogen peroxide solution. The production of gas bubbles in the test tube was taken as a positive reaction.

2) Coagulase test:

Slide coagulase:

A drop of saline was placed on a clean glass slide. The colony was emulsified in saline until a milky solution was formed. A drop of plasma was added to this bacterial suspension and stirred well. A positive result was interpreted when clumping was visible to naked eye.

Tube Coagulase:

1: 6 dilution of plasma was prepared in saline and 0.5 ml of the diluted plasma was taken in a small test tube. Few colonies from 18-24 hours culture of the test strain were added, incubated at 37^0C in water bath and examined for coagulum formation at 1, 2 and 4 hours. If no clot was seen, it was incubated overnight at room temperature and observed for clot formation. A visible clot was taken as a positive test.

3) Mannitol salt agar growth:

The colonies were streaked on a plate of mannitol salt agar and incubated at 37^0C for 16-24 hours. The presence of yellow colonies indicated that mannitol was fermented.

4) Urease test:

Colonies were picked up with a sterile loop and streaked on to Christensen's urease agar slope. It was incubated at 18-24 hours at 37^0C. Change in the color of the medium to pink indicated that the urea was hydrolyzed.

5) Sugar fermentation test:

A loop full of a 24 hour broth culture of the test organism was inoculated into tubes containing peptone water & sugars with bromothymol blue indicator and also inverted Durham's tube for detection of gas. Sugars used were Glucose, Maltose, Sucrose, Mannitol, Arabinose, Trehalose, Xylose, Raffinose and Cellobiose. The tubes were incubated at 37^0C for 24 hours. Change in color of the medium to yellow indicated fermentation of that sugar and air in Durham's tube indicated gas production.

All the above tests were performed in conjunction with positive, negative and test controls as necessary according to the standard guidelines.

GRAM NEGATIVE BACTERIA (GNB):

1. Catalase test:

An isolated colony from culture to be tested was picked up with a sterile glass rod and inserted into a test tube containing 3% hydrogen peroxide solution. The production of gas bubbles in the test tube was taken as a positive reaction.

2. Oxidase test:

Using oxidase disk containing 1% tetramethyl-para-phenylene diamine dihydrochloride reagent, the colonies were picked up with a glass rod and rubbed on it. Controls were made. A purple color formation indicated a positive reaction.

3. IMViC test:

Indole test:

The isolated Colonies were inoculated into peptone water and incubated for 18-24 hours. Then 0.5ml Kovac's reagent (p-dimethyl amino benzaldehyde, pure amyl or isoamyl alcohol, conc.HCl) was added. A pink color ring at the top of the solution indicated a positive test.

Methyl red test (MR):

Colonies were inoculated in glucose phosphate broth and incubated at 37^{o}C for 48 hours. A few drops of methyl red reagent were added. The development of a bright red color indicated a positive test.

Voges-Proskauer test (VP):

Colonies were inoculated in glucose phosphate broth and incubated at 37^{0}C for 48 hours. After 48 hours 6 drops of 5% solution of α-naphthol and 2 drops of potassium hydroxide solution were added. Shake well after the addition of reagent and observe for 5 minutes. The development of a red color indicated as positive test.

Citrate test:

Colonies were picked up with a sterile loop and streaked on to Simmon's citrate agar slope. It was incubated at 18- 24 hours at 37^{0}C. Change in the color of the media to blue indicated that the citrate was utilized.

4. Urease test:

Colonies were picked up with a sterile loop and streaked on to Christensen's urease agar slope. It was incubated at 18-24 hours at 37^{0}C. A change in the color of the media to pink indicated that the urea was hydrolyzed.

5. Triple sugar iron agar (TSI):

Colonies were picked up with a sterile straight wire and stabbed in to the media (butt) and streaked on the slant. It was incubated at 18-24 hours at 37^0C. The presence of a yellow color in both the slant and butt (Acid/ Acid - A/A) indicated that the organism was a lactose fermenter. Pink colored slant/ yellow colour butt (Alkali/ Acid - K/ A) indicated that the organism was a non-lactose fermenter. No change in the media indicated the organism was a Non-fermenter. Black color in the butt indicated H_2S production.

6. Mannitol motility medium test (MMM):

Colonies were picked up with a sterile straight wire and stabbed on to mannitol motility medium. It was incubated for 18- 24 hours at 37^0C. The change in the color of the media to yellow indicated mannitol fermentation and a haziness in the media indicated that the organism was motile.

7. Nitrate reduction test:

Colonies were inoculated into nitrate broth and incubated at 37^0C for 48 hours. 0.1ml test reagent (equal volume 0.8% Sulphanilic acid and 0.5% α-naphthylamine) was added. The development of red color in 2-5 minutes indicated a positive test.

8. Oxidation Fermentation test:

Colonies were picked up with a straight wire and inoculated in to 2 Hugh and Leiffson's media and one of the tubes was covered with liquid paraffin incubated at 37^0C. Yellow color change in both the tubes indicated that the organism was oxidative and fermentative. Yellow color change in the media in the tube not covered with paraffin and no change in the other media covered with paraffin indicated that the organism was oxidative only.

9. MOELLER'S DECARBOXYLASE TEST:

Prepared suspension of brain-heart infusion broth from a 24 hours old culture was inoculated in to each of three decarboxylase broths (lysine, arginine and ornithine) and control broth and 4-mm layer of sterile mineral oil to each tube was added and incubated at 37^0C in ambient air. The tubes were examined at 24, 48, 72 and 96 hours. Purple (Alkaline) color change observed when compared with control tube was considered as positive.

9. Sugar Fermentation Test:

A loop full of 24 hour broth culture of the test organism was inoculated into tubes containing peptone water with bromothymol blue indicator and also inverted Durham's tube. Sugars (Glucose, Sucrose, Lactose, Maltose, Mannose, Xylose, Trehalose and Arabinose) to be tested were added separately to each tube. The tubes were incubated at 37^0C overnight. A change in color of

the medium to yellow indicated fermentation of that sugar and air in Durham's tube indicated gas production.

All the tests above were performed in conjunction with positive, negative and test controls as necessary according to the standard guidelines.

Antimicrobial susceptibility testing:[33]

The isolated organism was subjected to antimicrobial susceptibility testing by disk diffusion method using modified Kirby-Bauer technique and interpreted as per CLSI 2016 guidelines. Three to four morphologically similar colonies were suspended in peptone water and incubated at 37^0C for 2 hours. The turbidity of the test suspension was standardized to 0.5 McFarland Units. The suspension was inoculated in Mueller Hinton agar plate with a sterile cotton wool swab by lawn culture method. After brief drying, antibiotic disks about 6 disks per 100 mm plate were placed with sterile precautions. Then the plate was incubated at 37°C for 24 hours and interpreted next day as per CLSI 2016, M100-S26, Volume 36, No.1.

Chart 5

PANEL OF DISKS USED FOR GRAM NEGATIVE BACILLI

Disk	Symbol	Drug concentration (µg)	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Gentamycin	G	10	≥15	13-14	≤12
Amikacin	AK	30	≥17	15-16	≤24
Ampicillin	A	10	≥17	14-16	≤13
Cotrimoxazole	CO	1.25/23.75	≥16	11-15	≤10
Nitrofurontoin	NF	300	≥17	15-16	≤14
Tetracycline	T	30	≥15	12-14	≤11
Norfloxacin	NA	300	≥17	13-16	≤12
Ciprofloxacin	CF	5	≥21	16-20	≤15
Cephelexin	CP	30	≥18	15-17	≤14
Cefotaxime	CE	30	≥26	23-25	≤22
Ceftriaxone	CI	30	≥21	14-20	≤13
Cefoxitin	CN	30	≥18	15-17	≤14
Ceftazidime	CA	30	≥18	15-17	≤14
Cefipime	CE	30	≥18	15-17	≤14
Aztreonam	AO	30	≥22	16-21	≤15
Amoxyclav	AC	20/10	≥18	14-17	≤13
Cefoperazone-Sulbactam	CFS	75/10	≥21	16-20	≤15
Piperacillin –Tazobactam	PT	100/10	≥21	18-20	≤21
Imipenam	I	10	≥23	20-22	≤19
Meropenam	M	10	≥23	20-22	≤19

Chart 6

PANEL OF DISKS USED FOR GRAM POSITIVE COCCI

Disk	Symbol	Drug Concentration (µg)	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Gentamycin	G	10	≥15	13-14	≤12
Amikacin	AK	30	≥17	15-16	≤24
Ampicillin	A	10	≥17	14-16	≤13
Cotrimoxazole	CO	1.25/23.75	≥16	11-15	≤10
Nitrofurontoin	NF	300	≥17	15-16	≤14
Tetracycline	T	30	≥15	12-14	≤11
Norfloxacin	NA	300	≥17	13-16	≤12
Ciprofloxacin	CF	5	≥21	16-20	≤15
Cephelexin	CP	30	≥18	15-17	≤14
Cefotaxime	CE	30	≥26	23-25	≤22
Ceftriaxone	CI	30	≥21	14-20	≤13
Cefoxitin	CN	30	≥18	15-17	≤14
Ceftazidime	CA	30	≥18	15-17	≤14
Cefipime	CE	30	≥18	15-17	≤14
Amoxyclav	AC	20/10	≥18	14-17	≤13
Vancomycin	V	30	≥15		≤14
Vancomycin	V	MIC	≤ 2	4-8	≥16

Detection of Vancomycin sensitivity for *Staphylococcus aureus* and *Coagulase negative staphylococcus* by Minimum Inhibitory Concentration technique by macrobroth dilution method:

1. Culture media- cation adjusted Mueller Hilton broth (pH-7.2 to 7.4)

2. Preparation of antibiotic stock solution

Vancomycin used for preparing stock solution was obtained from Macleods. Antibiotic Stock solution was prepared using the formula

$$W = \frac{1000}{\mathrm{P}} \times V \times C$$

where, P = potency of the antibiotic in relation to the base (for vancomycin potency will be 90%, not 100%)

$$\text{Potency of drug} = \frac{1000}{100} \times 90$$

$$= 900\mu g/ml$$

V= Volume of the stock solution to be prepared (10ml)

C= final concentration of the antibiotic solution (1024 microliter/ml)

W= weight of the antibiotic to be dissolved in the volume V

11.38 mg of drug was mixed with 10 ml of distilled water which contains 1024 micrograms/ml concentration of the drug.

3. Preparation of antibiotic dilutions

- Two rows each of 14 sterile test tubes were arranged in the rack (1st row for the test and 2nd row for ATCC control)

-Using Micropipette 1ml of Mueller Hinton broth was transferred to all the test tubes in the rack

-From the stock solution 1ml was transferred to the first tube in each row and mixed well

-From the first tube the 1ml of the antibiotic solution was transferred to the second tube

-This procedure was repeated till the end of 14th tube

-One tube containing only the antibiotic solution was kept as control

4. Inoculum preparation for the test and ATCC control

-9.9 ml of Mueller Hinton broth was taken in a sterile test tube

-0.1 ml of 0.5 McFarland turbidity matched test organism was added to broth and mixed well

-From the above inoculum 1 ml was transferred to each tube containing antibiotic dilutions

-One tube containing test inoculum was kept as control

-This procedure was repeated for ATCC control strain

5. Incubation

-the test tubes were incubated at 37^0 C for 24 hours

INTERPRETATION

-MIC of ATCC control strain and the test organism was observed

-The lowest concentration of the antibiotic which showed clearing was considered as the MIC for the ATCC strain and for the test isolate

Chart 7

Vancomycin MIC by Broth Macrodilution method

Organism	**Sensitive (µg/ml)**	**Intermediate Sensitive (µg/ml)**	**Resistant (µg/ml)**
Staphylococcus aureus	≤ 2	4-8	≥16
Coagulase negative staphylococcus	≤ 4	8-16	≥32

ANTIMICROBIAL RESISTANCE DETECTION METHODS:

a. **Methicillin Resistant- *Staphylococcus aureus* (MRSA), *CoNS***

b. Extended Spectrum beta lactamases (ESBL)

c. AmpC β-lactamases (AmpC)

a. Methicillin Resistant -*Staphylococcus aureus, CoNS*:[39]

Identification of *Staphylococcus aureus* and CoNS were done by the morphology of the colonies, pigment production, catalase test, slide and tube coagulase test, urease test and mannitol fermentation.

Methicillin resistance was identified by cefoxitin disk diffusion method. Three to four morphologically similar colonies were suspended in peptone water and incubated at 37ºC for 2 hours. The turbidity of the test suspension was standardized to 0.5 McFarland Units. The suspension was inoculated in cationic adjusted Mueller Hinton agar plate with a sterile cotton wool swab by lawn culture method. After brief drying, the antibiotic disk cefoxitin (30ug) was kept in the culture plate and incubated overnight at 37^0C. The test isolates were put up with necessary control strains to check the quality control of the method. The isolates with a zone of inhibition less than 21mm were reported as MRSA. For *Coagulase negative Staphylococcus* zone of inhibition less than 24mm were reported as Methicillin resistant.

Chart 8

Interpretation Methicillin resistant *Staphylococcus aureus, CoNS*

Organism	Methicillin Sensitive	Methicillin Resistant
Staphylococcus aureus	≥ 22mm	≤ 21mm
Coagulase negative staphylococcus	≥ 25 mm	≤ 24 mm

b.ESBL detection:[33],[41]

Isolates that showed resistant zone of inhibition to third generation cephalosporins i.e. ceftazidime (30µg) and cefotaxime (30µg) and to fourth generation cephalosporin, cefepime (30µg) were screened for ESBL production.

ESBL producing isolates were characterized phenotypically for ESBL production using double disk synergy test (DDST) as recommended by the Clinical Laboratory Standards Institute (CLSI). The test was done by using both ceftazidime (30µg) and ceftotaxime (30µg), alone and with ceftazidime-clavulanic acid and cefotaxime- clavulanic acid. More than 5 mm increase in zone diameter for the antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was taken as a positive result for ESBL production.

E-test for ESBL:[32][33]

Combination of disk diffusion and Minimum Inhibitory Concentration (MIC) were studied using the E-test strips. The E-test strip contains ceftazidime gradient at one end and ceftazidime plus clavulanic acid gradient on the opposite end. The organism that exhibited resistance to ceftazidime was swabbed onto a Mueller Hinton agar plate and the strip was placed on the inoculated lawn culture and incubated at 37^0C for 24 hours. MIC was the point of intersection of the inhibition ellipse with the E-test strip edge. Ratio of ceftazidime MIC and ceftazidime-clavulanic acid MIC of ≥ 8 indicated the presence of ESBL.

Quality Control (QC) used for ESBL production:

Escherichia coli ATCC 25922 - Negative control

Klebsiella pneumoniae ATCC 700603 – Positive control

C. AmpC Beta- Lactamase detection:

(a) Disk Antagonism test[35]

The organisms that exhibited resistance to third generation cephalosporins and cefoxitin were swabbed onto a Mueller Hinton Agar Plate and cefoxitin (30μg) and Ceftazidime (30μg) disks were placed at a distance of 20mm and incubated overnight at 37^0C. If the organism was Amp C β-

lactamases producer the AmpC β-latamases inducibility was recognized by blunting of the ceftazidime zone adjacent to cefoxitin disk.[35]

b. Amp C disk test [35][34]

The test is based on the use of Tris-EDTA to make the bacterial cell permeable and release β-lactamases into the external environment. AmpC disks (i.e., filter paper disks containing Tris-EDTA) were prepared in house by applying a mixture of saline and Tris -EDTA of 20µl in 1:1 ratio to sterile filter paper disks, allowed to dry and stored at 2-8^{0}C. The surface of a MHA plate was swabbed with a lawn of cefoxitin susceptible *E. coli* ATCC 25922 according to the standard disk diffusion method. Immediately prior to use, AmpC disks were rehydrated with 20 µl of saline and several colonies of each test organism were applied to a disk.

A 30µg cefoxitin disk was placed on the inoculated surface of the MHA. The inoculated AmpC disk was then placed almost touching the cefoxitin disk with the inoculated disk face in contact with the agar surface. The plate was then inverted and incubated overnight at 35°C in ambient air. After incubation, plates were examined for either an indention or a flattening of the zone of inhibition, indicating significant inactivation of cefoxitin (positive result) or the absence of a distortion, indicating no significant inactivation of cefoxitin (negative result).

DETERMINATION OF BIOFILM PRODUCING PROPERTY(42):

Detection of biofilm production in all the isolates was done by the following methods:

1. Congo Red Agar method (CRA)

2. Tube method (TM)

3. Tissue Culture Plate method (TCP)

CONGO RED AGAR METHOD:[(42)]

This is a qualitative method for detection of biofilm production and the medium used was Congo red agar (CRA) medium.

Procedure:

The medium composed of Brain heart infusion broth (37gm/l), sucrose (5gm/l), agar (10gm/l) and Congo red stain was prepared as a concentrated aqueous solution and autoclaved at 121ºC for 15 minutes. Then it was added to autoclaved Brain heart infusion agar with sucrose at 55ºC. Plates were inoculated with the test organism and incubated aerobically at 37°C for 24 to 48 hours.

Chart 9

Interpretation: Grading of biofilm producer by Congo red method

Biofilm producer	Colony morphology
High	Colonies with black color and a dry crystalline consistency
Moderate	Darkening of the colonies without dry crystalline consistency
Weak/Non-biofilm producers	Pink colored colonies.

TUBE METHOD:[42]

This is also a qualitative method for the detection of biofilm production.

Procedure:

1. The test organisms were inoculated in a 10 ml of trypticase soy broth taken in sterile test tubes. The tubes were kept for incubation.
2. Then the tubes were decanted and by using the phosphate buffer saline (pH 7.3), the tubes were washed and then it was allowed to dry.
3. By using 0.1% Crystal violet the tubes were stained and the deionized water was used to remove the excess stain.
4. The tubes were kept in an inverted position and allowed to dry. The control strains were included in the test and the scoring was done based on the results obtained.

Interpretation:

Biofilm production:

- A visible film lined the wall and the bottom of the tube.
- The amount of biofilm formed was scored as 1-weak/none, 2-moderate, 3-strong.

TISSUE CULTURE PLATE METHOD:[42]

This is a quantitative method for detection of biofilm formation.

Procedure:

1 The test organisms were inoculated in a 10 ml of trypticase soy broth and incubated for 24 hours.

2 A dilution of 1:100 was done for the cultures by using fresh trypticase soy broth medium. Add 200µl of the diluted cultures into the individual wells of a sterile 96 well flat bottom polystyrene tissue culture and then it was incubated.

3 Then the organisms used for controls (Positive control-*Staphylococcus epidermidis* ATCC 35984 (biofilm producer) and Negative control - *Staphylococcus epidermidis* ATCC 12228 (biofilm nonproducer) were diluted and added to the microtitre plate and kept for incubation.

4 Then gentle tapping was done to remove the contents of the well

5 Washing of the wells was done with 0.2 ml of phosphate buffer saline (pH 7.2) and then the wells were washed four times to remove the free floating bacteria.

6 After washing, 2% sodium acetate was used to fix the adherent bacteria in the wells and by using crystal violet (0.1%) the wells were stained and the deionized water was added to remove the excess stain and then allowed to dry for 20 minutes.

7 Reading was taken at a wavelength of 570nm by ELISA autoreader. The bacteria form a biofilm and adhere to the wells and the OD values were taken as an index of bacterial adherence to the wells.

Chart 10

Interpretation: Mean OD values for biofilm producer

Mean OD values	Biofilm production
<0.120	Non or weak biofilm producer
0.120-0.240	Moderate biofilm producer
>0.240	High bofilm producer

OD cut off value = average of negative control, +3x standard deviation (SD) of negative control

RESULTS

RESULTS

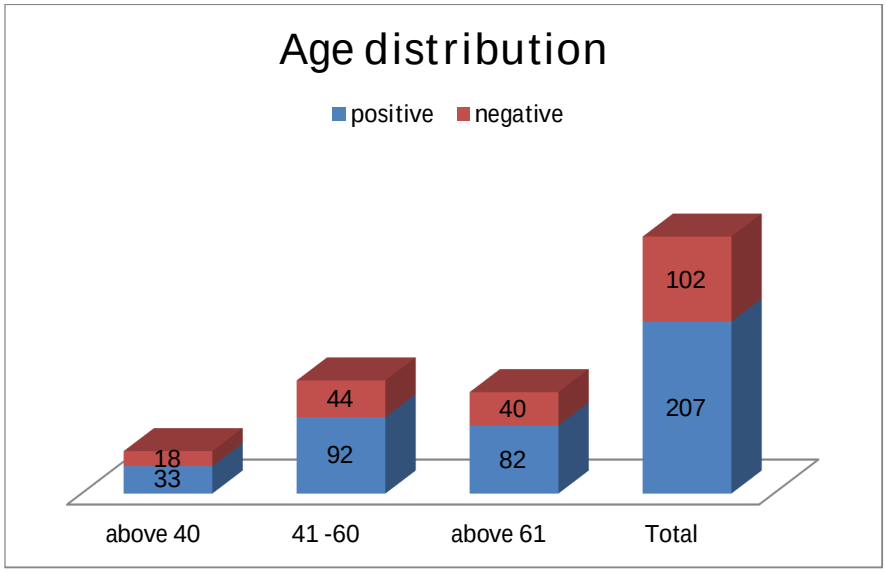
In this study totally 309 urine samples were collected from the patient with indewelling urinary catheters, with the symptoms of urinary tract infection in the Department of urology at Chengalpattu medical college. This study includes both the gender of age group above 18 years. Out of 309 sample 207(67%) showed growth and 102(33%) did not show any growth.

Table-1

Age wise distribution of CAUTI

Age wise distribution	CAUTI Positive	CAUTI Negative	Total
Up to 40years (n =51)	33 (64.7%)	18 (35.3%)	51 (100%)
41 – 60years (n =136)	92 (67.6%)	44 (32.4%)	136 (100%)
Above 61years (n =122)	82 (67.2%)	40 (32.8%)	122 (100%)
Total (n =309)	207 (67.0%)	102 (33.0%)	309 (100%)

Figure 1:



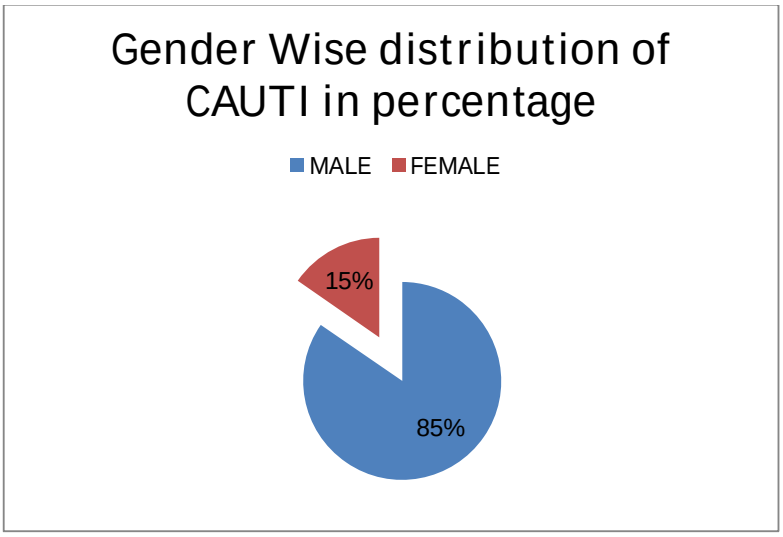
Among the 309 samples, the age group below 40 years were 51 and CAUTI positivity were 33(64.7%), the age group from 41 to 60 year were 136 and CAUTI positivity were 92(67.6%), the age group above 61 years were 122 and CAUTI positivity in them were 82(67.2%).

Table –2

Gender wise distribution of CAUTI (n=207)

Sex	CAUTI Positive
Male	175(85%)
Female	32(15%)

Figure 2:



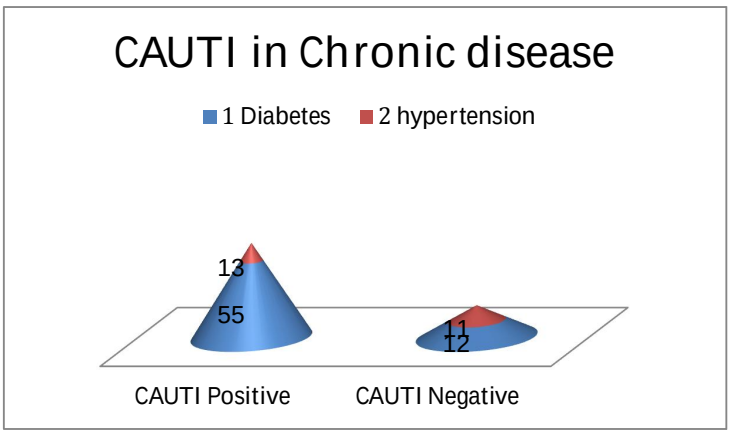
Among 207 CAUTI positive samples, the proportion of male was 175(85%) and female was 32(15%)

Table –3

CAUTI in chronic disease

Sl.no	Disease	CAUTI Positive	CAUTI Negative
1	Diabetes(n=67)	55(82%)	12(18%)
2	Hypertension(n=24)	13(54%)	11(46%)
3	Others(n=2)	0	2(100%)
4	Total(n=93)	68(73%)	25(27%)

Figure 3:



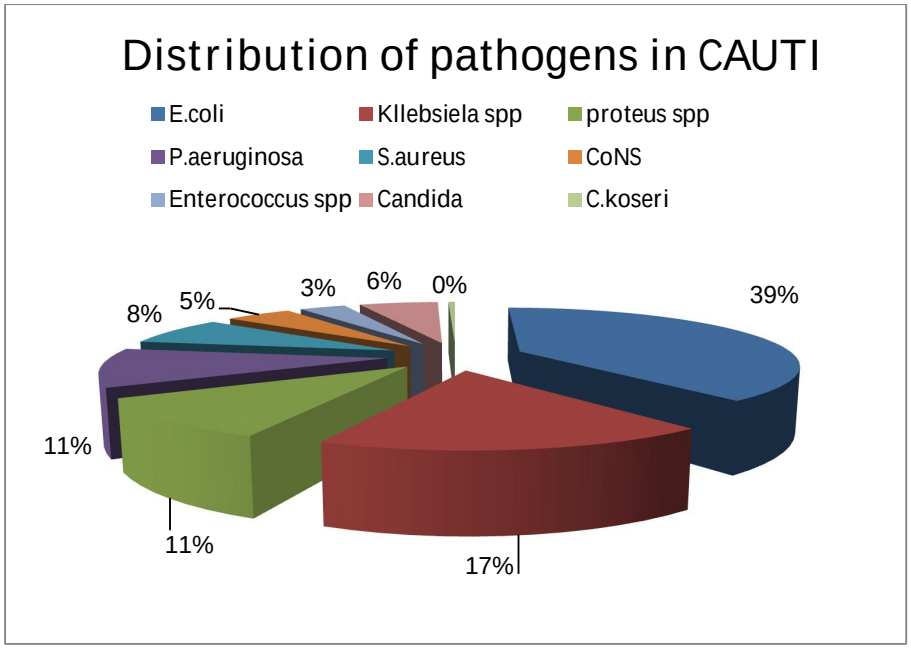
Out of 207 culture positive cases, 93 patients were having chronic diseases. In 93 patients, 67 were diabetics and 24 were hypertensive. Among the 67 diabetic patients, 55(82%) were culture positive. Where as in 24 hypertensive patients 13(54%) showed culture positive. Tuberculosis and hepatitis positive patient were negative for CAUTI.

Table-4

Distribution of pathogen in CAUTI

Organism	Frequency(n=207)	Percentage
Escherichia coli	81	39.13
Klebsiella spp.	36	17.39
Proteus spp	22	10.63
Pseudomonas aeruginosa	22	10.63
Citerobacter koseri	01	0.48
Staphylococcus aureus	16	7.73
CoNS	10	4.83
Enterococcus spp.	07	3.38
Candida	12	5.80
Total	207	100.00

Figure 4:



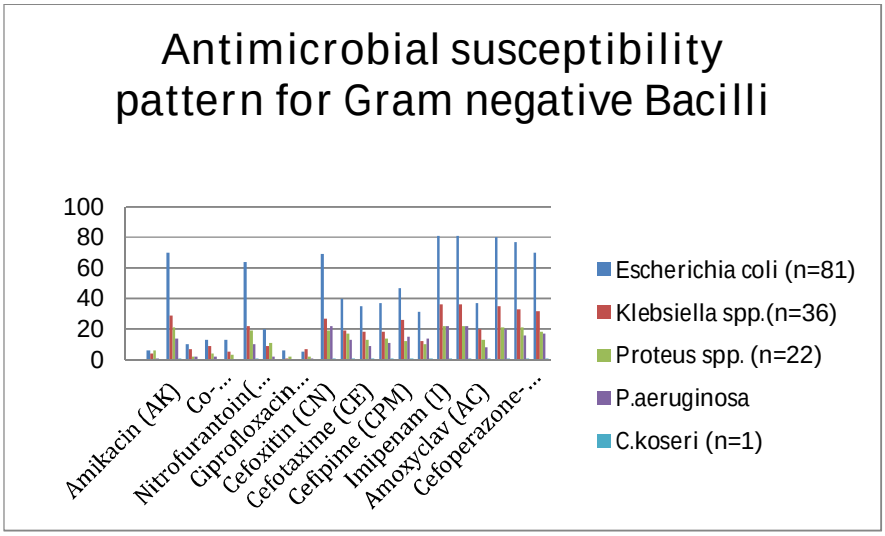
Of the 309 samples, 207 were culture positive. Among the 207 isolates the Gram negative bacilli (GNB) were 162 (78.2%) which was the major cause for CAUTI. The most common organism in GNB was *Escherichia coli* 81(39.13%) followed by *Klebsiella spp*. 36(17.39%), *Proteus spp*. 22(10.63%), *Pseudomonas aeruginosa* 22(10.63%) and *Citrobacter koseri* 01(0.48%). The Gram positive cocci was in 33 (15.94%) patients in which *Staphylococcus aureus* 16(7.73%) was the most common organism followed by *CoNS* 10(4.83%) and *Enterococcus spp*. 07(3.38%). *Candida* isolated in this study was 12 (5.80%).

Table -5

Antimicrobial susceptibility pattern for Gram negative Bacilli

Drugs	*Escherichia coli* (n=81)	*Klebsiella spp.*(n=36)	*Proteus spp.* (n=22)	*P.aeruginosa (n*=22)	*C.koseri* (n=1)	Total (n=162)
Gentamycin (G)	6(7%)	4(11%)	6(27%)	1(4%)	0	17(11%)
Amikacin (AK)	70(86%)	29(19%)	21(96%)	14(64%)	1(100%)	135(83%)
Ampicillin (A)	10(12%)	7(19%)	2(9%)	2(9%)	0	21(13%)
Co-trimoxazole(CO)	13(16%)	9(16%)	4(18%)	2(9%)	0	28(17%)
Norfloxacin (NX)	13(16%)	5(13%)	3(14%)	0	0	21(13%)
Nitrofurantoin(NF)	64(79%)	22(61%)	19(86%)	10(45%)	1(100%)	116(72%)
Ofloxacin(OF)	20(25%)	9(25%)	11(50%)	2(9%)	0	42(26%)
Ciprofloxacin (CF)	6(7%)	1(3%)	2(9%)	0	0	09(6%)
Cephalexin (CH)	5(6%)	7(19%)	2(9%)	1(5%)	0	15(9%)
Cefoxitin (CN)	69(85%)	27(75%)	19(86%)	22(100%)	1(100%)	138(85%)
Ceftazidime (CA)	40(49%)	19(53%)	17(77%)	13(59%)	1(100%)	90(56%)
Cefotaxime (CE)	35(43%)	18(50%)	13(59%)	9(41%)	1(100%)	76(47%)
Ceftriaxone (CI)	37(46%)	18(50%)	14(64%)	11(50%)	1(100%)	81(50%)
Cefipime (CPM)	47(58%)	26(72%)	12(55%)	15(68%)	1(100%)	101(62%)
Aztreonam (AO)	31(38%)	12(33%)	10(45%)	14(64%)	1(100%)	68(42%)
Imipenam (I)	81(100%)	36(100%)	22(100%)	22(100%)	1(100%)	162(100%)
Meropenan(Me)	81(100%)	36(100%)	22(100%)	22(100%)	1(100%)	162(100%)
Amoxyclav (AC)	37(46%)	20(56%)	13(59%)	8(36%)	1(100%)	79(49%)
Piperacillin-Tazobactam(PT)	80(99%)	35(97%)	21(96%)	20(91%)	1(100%)	157(97%)
Cefoperazone-Sulbactam(CFS)	77(95%)	33(92%)	21(96%)	16(73%)	1(100%)	148(91%)
Tetracycline(Tr)	70(86%)	32(89)	18(82%)	17(77%)	1(100%)	138(85%)

Figure 5 :



Out of 207 culture positive, 195(94%) were bacterial growth and 12(6%) were candida. The 195 samples were processed for drug sensitivity. In 195 samples, 162(78%) were Gram negative bacilli such as *Escherichia coli, Klebsiella spp., Proteus spp., Pseudomonas aeruginosa and Citrobacter koseri* which showed higher sensitivity to imipenam 162 (100%) and meropenam 162(100%) followed by piperacillin-tazobactam 157(97%), cefoperazone-Sulbactam 148(91%), tetracycline 38(85%), cefoxitin 138(85%), amikacin 135(83%), nitrofurantoin 116(72%) and cefipime 101(62%). Lower sensitivity pattern observed in ciprofloxacin 9(6%), cephalexin 15(9%), gentamycin 17(11%), norfloxacin 21(13%), ampicillin 21(13%), co-trimoxazole 28(17%) and ofloxacin 42 (26%).

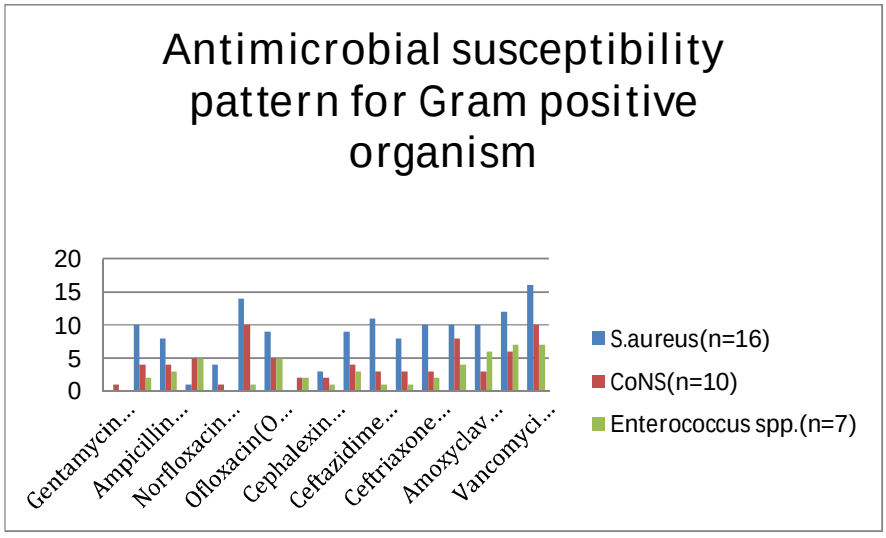
The isolates *Escherichia coli, Kiebsiella spp., Proteus spp*. which showed resistance to third generation cephalosporins were screened for ESBL production and AmpC β-lactamase production.

Table - 6

Antimicrobial susceptibility pattern for Gram positive organism

Sl.no	Drugs	*S.aureus*(n=16)	*CoNS*(n=10)	*Enterococcus spp.*(n=7)	Total(n=33)
1	Gentamycin (G)	0	1(10%)	0	1(3%)
2	Amikacin (AK)	10(62%)	4(40%)	2(29%)	14(42%)
3	Ampicillin (A)	8(50%)	4(40%)	3(43%)	15(45%)
4	Co-trimoxazole(CO)	1(6%)	5(50%)	5(71%)	11(33%)
5	Norfloxacin (NX)	4(25%)	1(10%)	0	5(15%)
6	Nitrofurantoin(NF)	14(87%)	10(100%)	1(14%)	25(76%)
7	Ofloxacin(OF)	9(56%)	5(50%)	5(71%)	19(58%)
8	Ciprofloxacin (CF)	0	2(20%)	2(29%)	4(12%)
9	Cephalexin (CH)	3(19%)	2(20%)	1(14%)	6(18%)
10	Cefoxitin (CN)	9(56%)	4(40%)	3(43%)	16(48%)
`11	Ceftazidime (CA)	11(69%)	3(30%)	1(14%)	15(45%)
12	Cefotaxime (CE)	8(50%)	3(30%)	1(14%)	12(36%)
13	Ceftriaxone (CI)	10(63%)	3(30%)	2(29%)	15(45%)
14	Cefipime (CPM)	10(63%)	8(80%)	4(57%)	22(67%)
15	Amoxyclav (AC)	10(63%)	3(30%)	6(86%)	19(58%)
16	Tetracycline(Tr)	12(75%)	6(60%)	7(100%)	25(76%)
17	Vancomycin (V)	16(100%)	10(100%)	7(100%)	33(100%)

Figure 6 :



Among the 195 bacterial isolates, 33(16%) were Gram positive cocci, (*Staphylococcus aureus, Coagulase negative Staphylococci, Enterococcus spp.)* showed higher sensitivity for vancomycin 33(100%) followed by tetracycline 25(76%), nitrofurantoin 25(76%), cefipime 22(67%), amoxyclav and ofloxacin 19(58%). The lower sensitivity observed in gentamycin 1(3%), ciprofloxacin 4(12%), norfloxacin 5(15%), cephalaxin 6(18%), co-trimoxazole 11(33%).

Table-7

Prevalence of Methicillin resistance

Sl.no	Organism	Methicillin Sensitive	Methicillin Resistance	Total %
1	*Staphylococcus aureus* (n=16)	9(56%)	7(44%)	100
2	*CoNS*(n=10)	4(40%)	6(60%)	100
3	Total(n=26)	13(50%)	13(50%)	100

Among 16 isolates of *Staphylococcus aureus,* 7(44%) were Methicillin resistant and among 10 isolates of *Coagulase negative staphylococci,* 6(60%) were Methicillin resistant. Total 26 isolates of GPC, 13(50%) were methicillin resistant.

Figure 7:

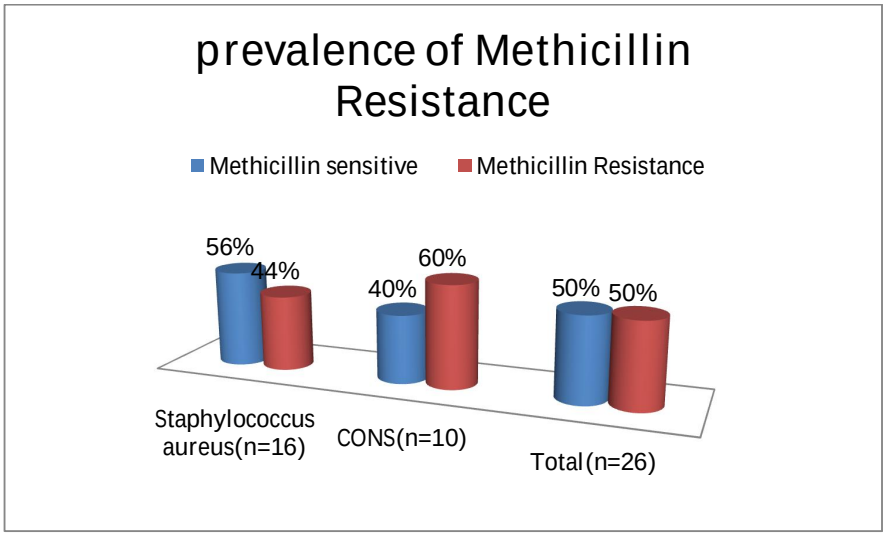
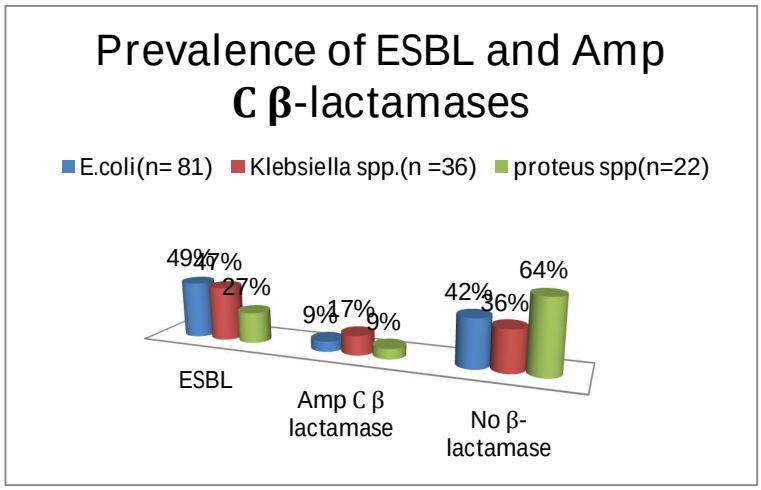


Table-8

Prevalence of ESBL and Amp C β-lactamases

Sl.no	Organism	ESBL	Amp C β – lactamase	No β-lactamase	Total (%)
1	*Escherichia coli* (n= 81)	40(49%)	7(9%)	34(42%)	100
2	*Klebsiella spp.* (n =36)	17(47%)	6(17%)	13(36%)	100
3	*Proteus spp* (n=22)	6(27%)	2(9%)	14(64%)	100
4	Total (n=139)	63(45%)	15(11%)	61(44%)	100

Figure 8:



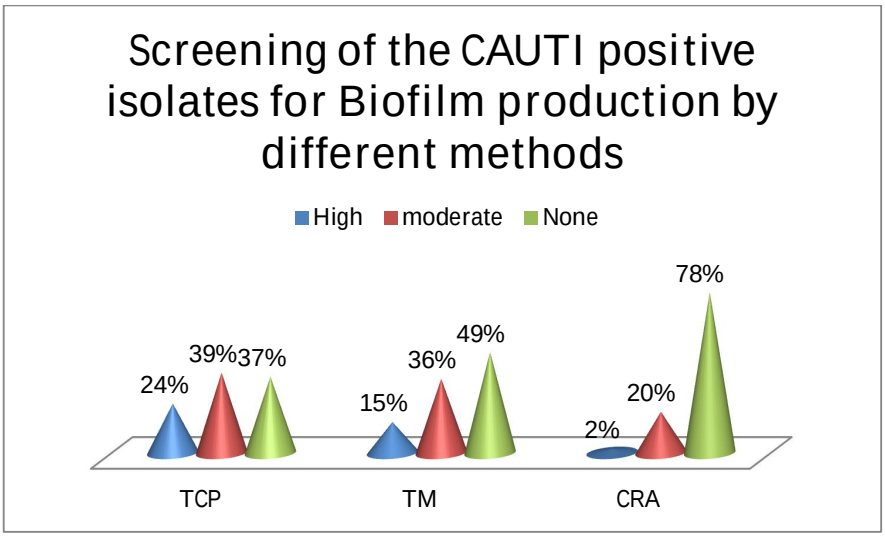
The phenotypic confirmation test and E test revealed that 40(49%) of *Escherichia coli,* 17(47%) of *Klebsiella spp.* and 6(27%) of *Proteus spp.* were found to be ESBL producers. The isolates were screened for Amp C β-lactamase production by Disk antagonism test, which showed 7(9%) *Escherichia coli,* 6(17%) *Klebsiella spp.* and 2(9%) *Proteus spp.* Among the 139 isolates (*Escherichia coli, Klebsiella* and *Proteus)* ESBL production was 63(45%) and AmpC β-lactamase was 15(11%).

Table -9

Screening of the CAUTI positive isolates for Biofilm production by different methods (n = 195)

	Biofilm production	TCP	TM	CRA
No.of isolates 195	High	47(24%)	29(15%)	4(2%)
	Moderate	75(39%)	71(36%)	38(20%)
	None	73(37%)	95(49%)	15(78%)
	Total	195(100%)	195(100%)	195(100%)

Figure 9:



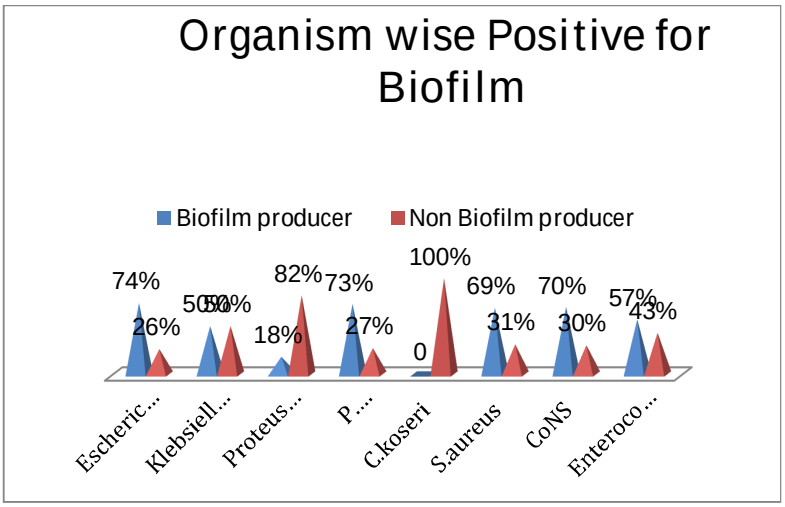
Out of 195 isolates, 122 (63%) were detected as biofilm producers by Tissue culture plate method. The Tube method detected 100(51%) isolates and Congo red agar method detected 42(22%) biofilm producing organisms.

Table-10

Organisms Positive for Biofilm

Sl.no	Organisms	Biofilm producer	Non Biofilm producer
1	*Escherichia coli*(n=81)	60(74%)	21(26%)
2	*Klebsiella spp.*(n=36)	18(50%)	18(50%)
3	*Proteus spp.*(n=22)	4(18%)	18(82%)
4	*Pseudomonas aeruginosa*(n=22)	16(73%)	6(27%)
5	*Citerobacter koseri*(n=1)	0	1(100%)
6	*Staphylococcus aureus*(n=16)	11(69%)	5(31%)
7	*CoNS*(n=10)	7(70%)	3(30%)
8	*Enterococcus spp.*(n=7)	4(57%)	3(43%)
9	Total(n=195)	120(62%)	74(38%)

Figure 10:



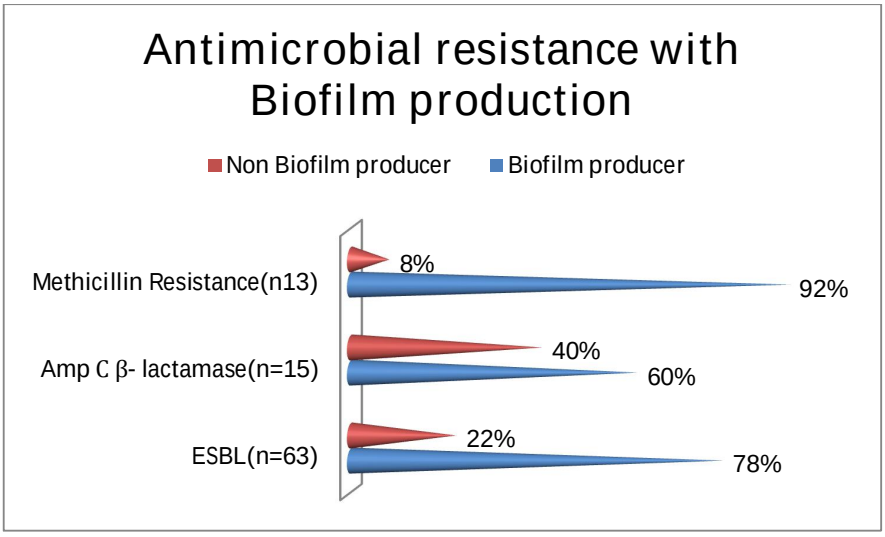
Among 195 isolates, 120(62%) were biofilm producers. Out of 81, 60 (74%) *Escherichia coli* were Biofilm producers followed by *Pseudomonas aeruginosa* 16(73%), *CoNS* 7(70%), *Staphylococcus aureus* 11(69%), *Enterococcus* 4(57%), *Kiebsiella* 18(50%) and *Proteus* 4(18%).

Table -11

Antimicrobial resistance with Biofilm production

Sl.no	Antimicrobial resistance	Biofilm producer	Non Biofilm producer
1	ESBL(n=63)	49(78%)	14(22%)
2	Amp C β- lactamase(n=15)	9(60%)	6(40%)
3	Methicillin Resistance(n=13)	12(92%)	1(8%)

Figure 11:



Out of 13 Methicillin resistant organisms, 12(92%) isolates were biofilm producers. Out of 63 ESBL producing organisms, 49(78%) isolates were Biofilm producers and out of 15 AmpC β-lactamase producers, 9 showed Biofilm production.

Table -12

Comparison of Tissue culture plate method with Tube method

Tube method	Tissue culture method		Total
	Positive	Negative	
Positive	98	2	100
Negative	24	71	95
Total	122	73	195

Table -13

Comparison of Tissue culture plate Method with Congo red Method

Congo red Method	Tissue culture method		Total
	Positive	Negative	
Positive	41	1	42
Negative	81	72	153
Total	122	73	195

Table -14

Comparison of Tube method with Tissue culture method

Tissue culture method	Tube method		Total
	Positive	Negative	
Positive	98	24	122
Negative	2	71	73
Total	100	95	195

Table -15

Comparison of Congo red method with Tissue culture Method

Tissue Culture Method	Congo red method		Total
	Positive	Negative	
Positive	41	81	122
Negative	1	72	73
Total	42	153	195

Table -16

Sensitivity and Specificity of TCP, TM and CRA methods

Methods	Sensitivity	Specificity	PPV	NPV
TCP	98%	74%	80%	97%
TM	80%	97%	98%	75%
CRA	34%	98%	97%	47%

IMAGES

IMAGE -1 : SAMPLE COLLECTION KIT

IMAGE -2 : SAMPLE COLLECTION TECHNIQUE

IMAGE -3: GRAM STAINING SHOWING PLENTY OF GRAM NEGATIVE BACILLI AND PUS CELLS

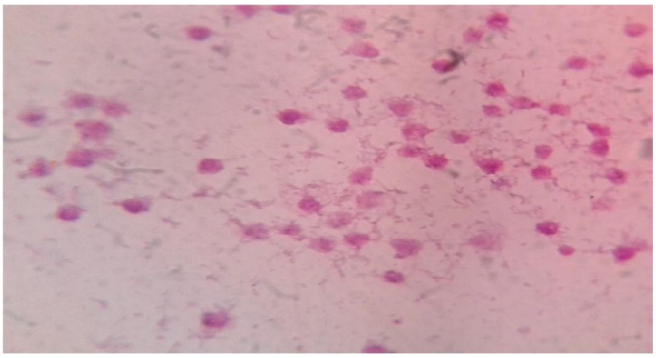


IMAGE -4 : SEMI QUANTITATIVE CULTURE IN CLED AGAR PLATE

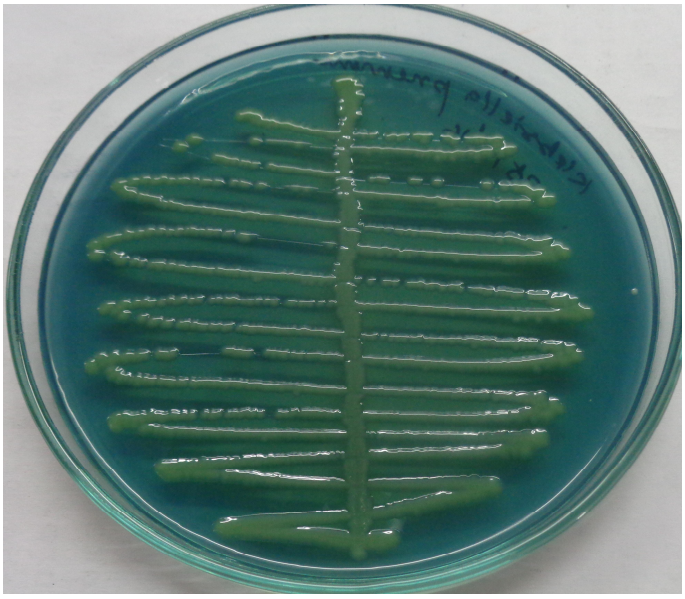


IMAGE -5 : GROWTH OF *STAPHYLOCOCCUS AUREUS* ON CLED AGAR PLATE

IMAGE -6 : MIC OF VANCOMYCIN MACRO BROTH DILUTION METHOD

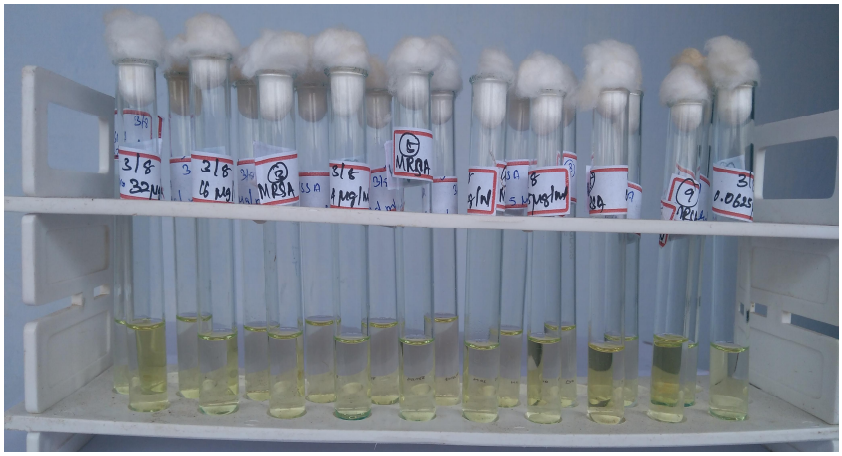


IMAGE -7 : BIOCHEMICAL REACTIONS FOR GRAM NEGATIVE BACILLI

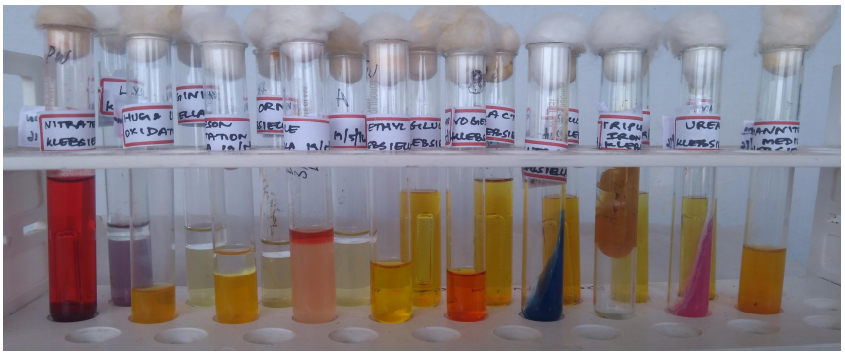
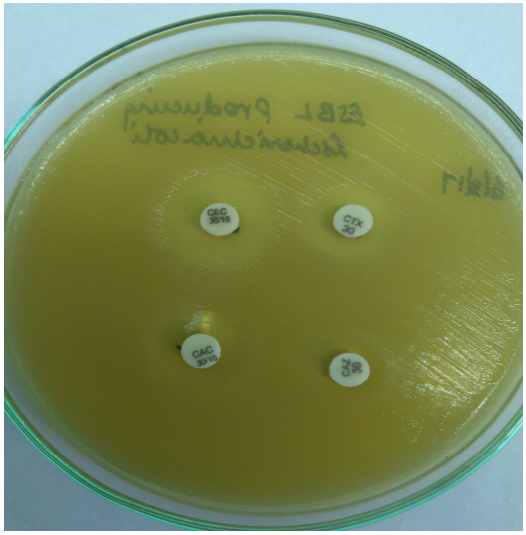
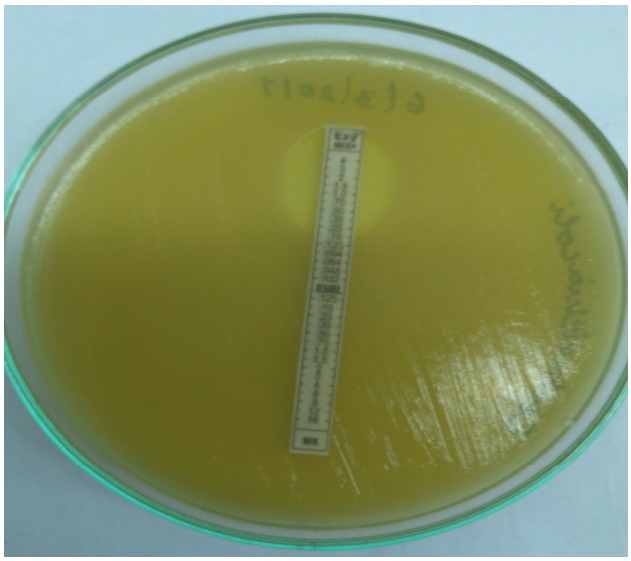


IMAGE -8 : PHENOTYPIC DETECTION METHOD FOR ESBL



More than 5mm increase in zone diameter for cefotaxime – clavulanic acid and ceftazidime- clavlanic acid when compared with cefotaxime and ceftazidime.

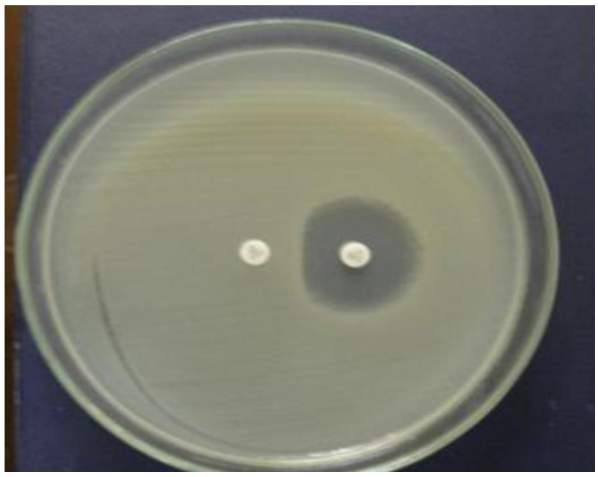
IMAGE - 9: E-TEST FOR ESBL



Ratio of ceftazidime MIC and ceftazidime clavulanic acid mix of > 8 indicates the presence of ESBL

IMAGE - 10: PHENOTYPIC DETECTION METHOD FOR AmpC β-LACTAMASE

Disk antagonism test



AmpC disk test

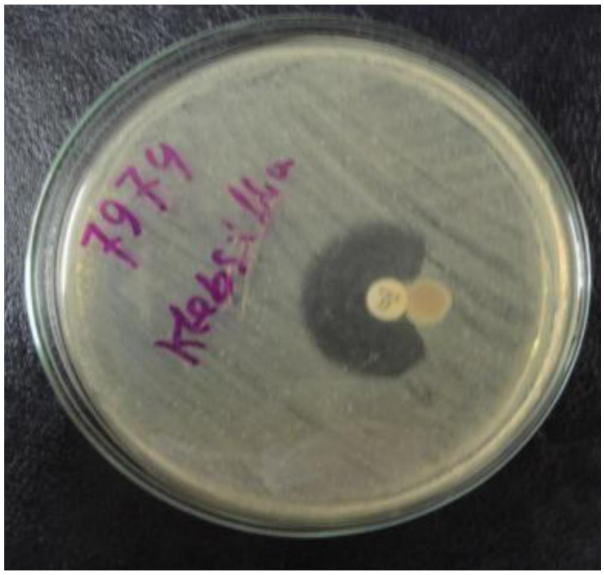
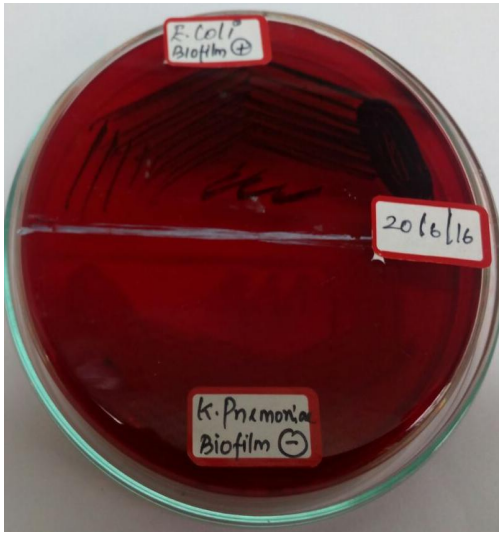


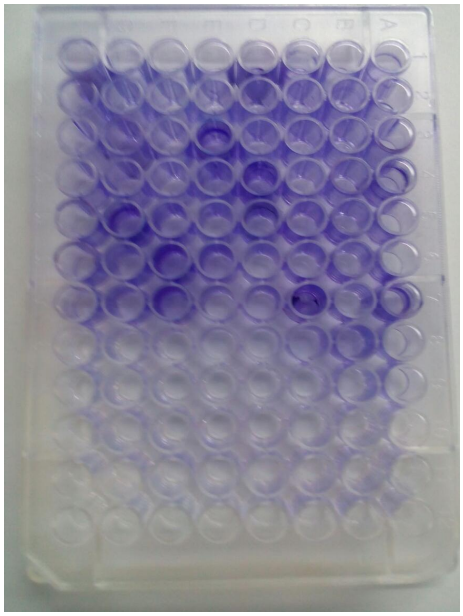
IMAGE -11 : BIOFILM DETECTION METHOD

CONGO RED METHOD



TUBE METHOD

TISSUE CULTURE PLATE METHOD



DISCUSSION

DISCUSSION

Urinary tract infection, its diverse clinical syndromes and affected host groups, remains one of the most common but widely misunderstood and challenging infectious disease encountered in clinical practice. The risk of developing urinary tract infection increases significantly with use of indwelling devices. Catheter in urinary tract disrupts normal host defence, resulting in over distension of the bladder and incomplete voiding that leaves residual urine for microbial growth favoring bacterial colonization. Due to introduction of foreign body, pathogens require few recognized virulence factors to colonize and establish infection than to infect fully functional urinary tract. Biofilm mode of growth has been well documented as one of the important cause of prosthetic device related infections that are refractory of treatment

In the present study, out of 309 urine samples processed, 207(67%) showed significant growth of organisms. The results were correlated with the study conducted by **Vinoth M et al**[43] that reported 70% of culture positivity. **Pallavi et al**[44] documented that out of 339 samples, 278 (82%) showed significant growth. However, **Teshager L et al**[45] and **Mohamed et al**[46] observed 43.3% and 53.3% of culture positivity in their study.

The present study, Catheter associated urinary tract infections for the age group 20-40 years were 64.7%, 41-60 years were 67.6% and from 61-90 years were 67.2%. The prevalence in all age group was almost equal and this

was correlated with the study conducted by **A.B.Mulhall et al**[47] who found that there were no association between age and risk of acquiring bacteriuria. Similarly **Jonson et al**[48] also found there were no association of Urinary tract infection with the age of patient. However **S.G.Kulkarni et al**[49] found that CAUTI was more common with increasing in age >40 years and **N.Bhatia et al**[50] also found that the occurrence of CAUTI was more common with increasing age. The reason for occurrence of UTI in older age may be due to age-associated changes in immune function, exposure to nosocomial pathogens and an increasing number of co-morbid conditions. All together put the elderly at an increased risk for developing infection. But in our study the occurrence of Catheter associated Urinary tract infection in all age group ranging from 20 to 90 years was same; this may be due to insitu catheter for prolonged period that is for more than 6 days.

The gender wise distribution of CAUTI in our study, Males were more affected 85% than females 15%. In contrary to other studies by **Mangukiya JD, et al**[51] said that females were more commonly affected. The increased risk of UTI in females was likely to be due to easier access of the perineal flora to the bladder along the outside of the catheter as it travels the shorter female urethra. In addition to it woman's urethra is closer to anus. The higher prevalence of CAUTI in male from our study was due to traumatic injury of genitourinary tract more in the age group of < 40 years male ultimately leads to prolonged catheterization. In the age group >40 years many were affected by

benign prostatic hypertrophy which was correlated with the study conducted by **Vinoth M et al[43][52][53].**

Among the chronic diseases, Diabetes (55%) was the common factor associated with urinary tract infection in our study. This was correlated with the study by **Eshwarappa et al[54][55]**. Diabetes mellitus produces a number of long-term effects on the genitourinary system. Diabetic nephropathy is one of the major factors that make these patients more susceptible to UTIs than the non-diabetics. The reduced immunity in diabetes also contributes to the increased risk for acquiring UTI.

Table 17 :

The distribution of Pathogen in our study was compared with other study

Sl.no	Organism	Present Study (%)	Pallavisayall [44] (%)	Mahim Koshariya[3] (%)	Niveditha [55] (%)
1	*Escherichia coli*	39.13	29.70	53.3	70
2	*Klebsiella spp.*	17.39	24.75	6.7	16
3	*Proteus spp*	10.63	16.83	8.8	
4	*Pseudomonas aeruginosa*	10.63	20.79	13.3	2
5	*C.koseri*	0.48	6.93	-	-
6	*Staphylococcus aureus*	7.73	10.61	13.3	-
7	*CoNS*	4.83		-	6
8	*Enterococcus spp.*	3.38		2.2	1
9	*Candida*	5.80	-	2.2	

In the present study, *Escherichia coli* (39.13%) was the commonest organism isolated which was followed by *Klebsiella spp* (17%). This was correlated with the study conducted by **Pallavisayall et al, Niveditha et al** and **MahimKoshariya et al**. The occurrence of *Proteus* (10.63%) and *Pseudomoas* (10.63%) in this study was almost equal with the study conducted by **Mahim Koshariya et al.** Among Gram positive organisms, *Staphylococcus aureus* (7.73%) was the commonest organism followed by *Coagulase negative staphylococcus* (4.83%) and *Enterococcus spp*. (3.38%) which totally constitute 16% .This was same with the study conducted by **Mahim Koshariya et al**, however it was higher when compared with **Pallavisayall et al** which showed only 10.61%.

Hedlund M et al[54] said that *Escherichia coli* was responsible for more than 80% of all the Urinary tract infections and it causes both symptomatic Urinary tract infection and asymptomatic bacteriuria. The ability of the Uropathogenic *Escherichia coli* to cause symptomatic UTI which was associated with the expression of a variety of virulence factors, which include adhesins (e.g., type 1 and P fimbriae) and toxins (e.g., haemolysin).

The most effective antibiotics against Gram negative organisms in our study were imipenam and meropenam (100%) followed by piperacillin-tazobactam (97%), cefoperazone-sulbactam (91%), tetracycline (85%), cefoxitin (85%), amikacin (83%), nitrofurantoin (72%) and cefipime (62%). This was correlated with the study by **Mahabubul Islam Majumder et al**[56]

and **Akram et al**[57] from India that has reported 100% susceptibility to the drug imipenam and similar findings were also reported by **Ullai et al**[58] from Pakistan. **Michael Osthoff et al**[59] in his study showed 100 % sensitive to the drug imipenam and 84% sensitive for the drug nitrofurantoin**. Sujatha et al**[60] in her study revealed that most of the Gram negative bacilli were sensitive to nitrofurantoin (97%), imipenam (95%), amikacin (85%), piperacillin-tazobactam (80%), cefoperazone-sulbactam (80%) which is contrary to our study.

Lower sensitivity pattern was observed for the drugs ciprofloxacin (6%), cephalexin (9%), norfloxacin (13%), ampicillin (13%) and co-trimoxazole (17%) in our study, which was correlated with the study conducted by **Sujatha et al** that showed higher resistance for the drugs ampicillin, fluoroquinolones and cephalosporins. This can be due to indiscriminate use of these drugs for all type of infections and occurrence of drug resistant mutants.

The studies from Europe, USA and many other countries had showed better susceptibility pattern for pathogens isolated from UTI against co-trimoxazole[61][62]. But, in our region co-trimoxazole had poor response[63]. A reason for this lack of sensitivity may be due to the drug co-trimoxazole had been extensively used. Among the 81 isolates of *Escherichia coli*, 68 (84%) strains were resistant to co-trimoxazole. Hence, co-trimoxazole cannot be recommended as an empiric therapy for the treatment of UTI in our area.

The drugs which showed higher sensitivity in our study were imipenam, meropenam, and also piperacillin-tazobactam, cefoperazone-sulbactam, tetracycline, cefoxitin, amikacin, nitrofurantoin would be better choice for the empric treatment for CAUTI in our Hospital.

Among the Gram positive organisms, the drug vancomycin (100%) followed by tetracycline (76%) and nitrofurantoin (76%) showed higher sensitivity which was same with the study conducted by **Zahra Tayebi et al.**[(64)] The lower sensitivity was observed for the drugs gentamycin (3%), ciprofloxacin (12%), norfloxacin (15%), cephalexin (18%) and co-trimoxazole (33%) which was similar with the study conducted by **Sujatha et al.**

High incidence of multi drug resistant strains was found in our study. It could be attributable to high usage of antimicrobials agents. Continued use of antibiotics for treatment of UTI should be supported by monitoring of antimicrobial susceptibility mainly to prevent the spread of resistant isolates and also to eliminate the use of antibiotics for a prolonged period.[(65)]

Based on the screening and confirmatory tests conducted to determine the total number of drug resistance, we observed out of 207 cultures positivity 26 were Gram positive organisms. Among this 26 isolates, 13(50%) were Methicillin resistant. Out of 139 Gram negative organisms (*Escherichia coli, Klebsiella spp., Proteus spp.)* 63 (45%) were ESBL producing organisms and 15(11%) AmpC β-lactamase producers. None of the *Klebsiella* were KPC

producers which was correlated with the study by **Shilpa et al** that showed 50% of Methicillin resistance and 67% were in combination of ESBL and AmpC β-lactamase producer.[62] However **Mehta et al**[66] in his study showed 74.1% was ESBL producer which is higher when compared with our study.

GouseBasha Sheik et al[67] in his study showed 85(56.7%) were ESBL producer and 24(16%) were AmpC β-lactamase producer. This is high when compared with our study. In a similar study, **Amreliwala et al**[68] reported that 52.5% of the isolates included in the study were ESBL producers and 22.25% were AmpC producers. In a study conducted by **Loveena et al**[69] found that, out of 273 Gram negative isolates, 96 (35.16%) were ESBL producers, followed by 15 (5.4%) were AmpC β-lactamase producers which is in contrast to our study.

The present study showed that 122 (63%) Uropathogens were biofilm producers detected by tissue culture plate method. Though many studies[70] had shown variable biofilm formation (45%-60%) among uropathogens. With respect to phenotypic biofilm detection methods, we screened isolates by Tissue culture plate method(TCP), Tube method(TM) and Congo red agar method(CRM) because of their ease of performance in routine laboratory settings for determining biofilm formation. Tissue culture plate method detected (63%) as biofilm producers. Out of which, 24% were high, 39% were moderate biofilm producers and 37% were non biofilm producers.

Table 18 :

Comparison of Biofilm detection method with other study

Year	Author	Biofilm detection method		
		TCM (%)	TM (%)	CRM (%)
2016	Present study	63	51	22
2015	Tayal R et al[71]	27%	37.96%	40.88%
2015	Mohamad E et al	45.6%	38.11%	36.9%
2014	NabajitDeka[42]	83	57	20
2011	Hassan et al[72]	63.63	54	11
2006	Turkyilmaz et al[73]	50.50	55.55	61.10

In the present study, the percentage of biofilm production by Tissue culture plate method (63%) was similar with study conducted by **Hassan et al.** Tube method (51%) was also similar with study conducted by **Hassan et al** and **Turkyilmaz et al**. The Congo red method detection of Biofilm production (22%) was similar with the study by **Nabajit Deka** and it was lesser when compared with the study by **Hassan et al**.

The most common organisms producing biofilm in our study were *Escherichia coli* (74%) *and Pseudomonas aeruginosa* (73%) which was correlated with the study by **Ghanwate et al** [74] and **Sayal et al**[44] were *Escherichia coli and Pseudomonas aeruginosa* was the common biofilm producing organism in their study.

All the isolated bacteria are the normal flora of human intestine voided in the faeces and are also present on the perianal region from where they may migrate to the genitals exterior and contaminate the catheter tip while inserting, if proper cleaning of the genitals or urethra is not done. From the tip of the catheter these bacteria may migrate to the lumen and establish them in the form of a biofilm. Another reason may be due to the open drainage system. These bacteria may travel up to the catheter opposite to the direction of urine flow and produce the biofilm. This explanation was given by **Stamm et al.**[75]

Higher antibiotic resistance was observed in biofilm producing strains, Methicillin resistance were 12(92%), Extended spectrum of β lactamase were 49(78%) and AmpC β-lactamase were 9(60%). This may be due to slow penetration of the drug, phenotypic resistance and altered micro environment. In our study biofilm producing organism shows higher antimicrobial resistance when compared with non biofilm producer which was similar with the study conducted by **Godalyet al.** [56]

Table 19:

Comparison of sensitivity and specificity of TM and CRM with other study

Author	Tube method		Congo red method	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**
Present study	80	97	34	98
Tayal R et al[71]	94.59	83.00	94.59	81.00
Hassan A et al[72]	73	92	11	92

Tissue culture method when compared with Tube method and Congo red method, shows higher sensitivity of 98%. Tissue culture method is the best screening method for detection of biofilm production. The tube method in our study was showing 80% sensitivity, 97% specificity. Based on our observations, the Congo red method was only 34% sensitive which was lower when compared with the study by **Tayal R et al** and the Congo red method in detection of biofilm was higher when compared with **Hassan A et al.** Based on this finding we don't recommend Congo red method as suitable for biofilm screening. Our results were in agreement with other investigators as shown in table 27.

SUMMARY

SUMMARY

The study was conducted at Chengalpattu Medical college Hospital, Chengalpattu, over a period of one year from March 2016 to February 2017 with 309 patients suffering from Catheter associated urinary tract infection. Among 309 patients, 207 (67%) had significant bacteriuria. Male preponderance had been noted in the current study. The occurrence of urinary tract infection was equal in all age groups from 20 to 90 years.

- Among the chronic diseases, Diabetes was the common factor associated with urinary tract infection in this study.

- In this study, out of 207 cultures positive, 162 were Gram Negative Bacilli and 33 were Gram Positive organism. Among which *Escherichia coli* 81(39.13%) was the commonest organism isolated followed by *Klebsiella spp.* 36(17.39%), *Proteus spp*. 22(10.63%), *Pseudomonas aeruginosa* 22(10.63%) and *Citrobacter koseri* 01(0.48%).

- Among the Gram positive cocci, 16(7.73%) were *Staphylococcus aureus*, 10(4.83%) were *CoNS* and 07(3.38%) were *Enterococcus spp*.

- *Candida* isolated in this study was 12 (5.80%).

- All the Gram negative bacilli were mostly sensitive to imepenam (100%), followed by piperacillin-tazobactam (97%), cefoperazone-

sulbactam (91%), tetracycline (85%), amikacin (83%) nitrofurantoin (72%), cefipime (62%).

- Among the Gram positive organisms, Vancomycin (100%) showed higher sensitivity followed by tetracycline (76%) and nitrofurantoin (76%)

- Prevalence of ESBL production in *Escherichia coli* was found to be 49%, for *Klebsiella spp* was 47% and *Proteus spp* was 27%.

- Prevalence of Amp C β lactamase production was 9% in *Escherichia coli*, 17% in *Klebsiella spp*. and 9% in *Proteus spp*.

- Prevalence of Methicillin resistance in *Staphylococcus aureus* was 44% and for *Coagulase negative staphylococcus* was found to be 60%.

- Phenotypic detection of biofilm production among catheter associated Urinary tract infection were high in Tissue culture plate method 122(63%) when compared with Tube method 100 (51%) and Congo red method 42(22%).

- Among the Biofilm producers, *Escherichia coli* (74%) was the most common organism followed by *Pseudomonas aeruginosa (73%), Coagulase negative staphylococcus (70%), Staphylococcus aureus (69%), Enterococcus spp. (57%), Klebsiella spp.*(50%) and *Proteus spp.(18%)*

- Antimicrobial resistance among the biofilm producers were Methicillin resistance organisms 12(92%), ESBL producing organisms 49(78%) and AmpC β-lactamase producers 9(60%).

- The sensitivity for detection of biofilm production by Tissue culture plate method was high (98%) when compared with Tube method (80%) and Congo red method (34%)

CONCLUSION

CONCLUSION

Based on our study, we conclude that, Gram negative organism shows higher sensitive to imepenam, it should be kept in reserve as the second line of drug. Other drugs which was most economic and orally effective like Nitrofurantoin can be given to outpatients. Amikacin, cefipime and β-lactamase inhibitors like piperacillin-tazobactam, cefeperazone-sulbatam can be given to inpatients.

Due to subjective variations in interpretation and lack of reproducibility among test results, tube method cannot be suggested as general screening test to identify biofilm producing isolates. Thus, we conclude from our study that TCP is a quantitative and reliable method to detect biofilm forming microorganisms. When compared to tube method and Congo red method, Tissue culture plate can be recommended as a general screening method for detection of biofilm producing bacteria in our laboratory.

BIBILOGRAPHY

1. Nicolle LE. Catheter associated urinary tract infections. Antimicrob Resist Infect Control [Internet]. 2014;3(1):23. Available from: http://aricjournal.biomedcentral.com/articles/10.1186/2047-2994-3-23

2. Soto SM. Importance of Biofilms in Urinary Tract Infections : New Therapeutic Approaches. 2014;2014.

3. Koshariya M, Songra MC, Namdeo SR, Chaudhary A, Agarwal S, Rai A. Prevalence of pathogens and their antimicrobial susceptibility in catheter associated urinary tract infection. 2015;2(4):96–113.

4. B1. F. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med. 2002;113(Suppl 1A):5S–13S.

5. Module D. Urinary Tract Infection (Catheter-Associated Urinary Tract Infection [CAUTI] and Non-Catheter-Associated Urinary Tract Infection [UTI]) and Other Urinary System Infection [USI]) Events. 2016;(January):1–16.

6. Anne M, Amalaradjou R. Role of Bacterial Biofilms in Catheter-Associated Urinary Tract Infections (CAUTI) and Strategies for Their Control.

7. Warren JW. Catheter-associated urinary tract infections. 2001;17: 299–303.

8. Warren JW. Catheter-associated urinary tract infections. Infect Dis Clin North Am. 1997 Sep;11(3):609–22.

9. Hospitals US. Estimating Health Care-Associated Infections and Deaths in. 2007;122(April):160–6.

10. Douglas AR, Ii S. The DirecT MeDical cosTs of and the Benefits of Prevention. 2009;(March).

11. Garibaldi, R. A., J. P. Burke, M. R. Britt, M. A. Miller and CBS. Meatal colonization and catheter-associated bacteriuria. N Engl J Med. 303:.:316–318.

12. Donlan RM, Costerton JW. Biofilms : Survival Mechanisms of Clinicall Relevant Microorganisms. 2002;15(2):167–93.

13. Karatan E, Watnick P. Signals , Regulatory Networks , and Materials That Build and Break Bacterial Biofilms. 2009;73(2):310–47.

14. Li X, Zhao H, Lockatell CV, Drachenberg CB, Johnson DE, Mobley HLT. Visualization of Proteus mirabilis within the Matrix of Urease-Induced Bladder Stones during Experimental Urinary Tract Infection. 2002;70(1):389–94.

15. Nickel JC, Olson M, McLean RJ, Grant SK CJ. An ecological study of in- fected urinary stone genesis in an animal model. Br J Urol. 1987;59(21–3):[107].

16. Bang CS, Kruse R, Johansson K, Persson K. Carbon monoxide releasing molecule-2 (CORM-2) inhibits growth of multidrug- resistant uropathogenic Escherichia coli in biofilm and following host cell colonization. BMC Microbiol [Internet]. 2016;1–10. Available from: http://dx.doi.org/10.1186/s12866-016-0678-7

17. Umscheid CA, Mitchell MD, Doshi JA. Estimating the Proportion of Healthcare- Associated Infections That Are Reasonably Preventable and the Related Mortality and ... Estimating the Proportion of Healthcare-Associated Infections That. 2013;(February 2011).

18. Jacobsen SM, Stickler DJ, Mobley HLT, Shirtliff ME. Complicated Catheter-Associated Urinary Tract Infections Due to Escherichia coli and Proteus mirabilis. 2008;21(1):26–59.

19. Jain P, JP P, David A, LG S. Overuse of the indwelling urinary tract catheter in hospitalized medical patients. Arch Intern Med [Internet]. 1995 Jul 10;155(13):1425–9. Available from: http://dx.doi.org/10.1001/archinte.1995.00430130115012220. OA P, LH W, VT F. Suprapubic bladder drainage in general surgery. Arch Surg [Internet]. 1973 Apr 1;106(4):568–72. Available from: http://dx.doi.org/10.1001/archsurg.1973.01350160180031

21. Kunin, C. M. Lea and Feniger, Philadelphia P. Detection, prevention, and management of urinary tract infections. Pubmed.

22. Cravens, D. D. and SZ. Urinary catheter management. Am Fam Physician. 61:369–376.

23. Maki, D. G., V. Knasinski, K. T. Halvorson, P. A. Tambyah and RGH. A prospective, randomized, investigator-blinded trial of a novel nitrofurazone-impregnated urinary catheter.. Infect Control Hosp Epidemiol. 1997;18:/50.

24. Darouiche, R. O., J. A. Smith, Jr., H. Hanna, C. B. Dhabuwala, M. S. Steiner, R. J. Babaian, T. B. Boone, P. T. Scardino, J. I. Thornby and IIR. Efficacy of antimicrobial-impregnated bladder catheters in reducing catheter-associated bacteriuria: a prospective, randomized, mul- ticenter clinical trial. Urology. 1999;54::976–981.

25. Walker S, Mcgeer A, Simor AE, Armstrong-evans M, Loeb M. Why are antibiotics prescribed for asymptomatic bacteriuria in institutionalized elderly people? 2000;163(3):273–7.

26. Edition T, Morse SA, Carroll KC. jawittz,26.

27. Mandell D and B. Principle and Practice of Infectious Disease 8th edition.

28. Brunton LL, Chabner BA, Knollmann BC. Goodman and gilman's the pharmacological basis of therapeutics. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 2011. 1808 p.

29. Hooton TM1, Scholes D, Stapleton AE, Roberts PL, Winter C, Gupta K, Samadpour M SW. A Prospective Study of Asymptomatic Bacteriuria in Sexually Active Young Women. N Engl J Med. 2000;343(14):992–7.

30. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev [Internet]. 2005 Oct 1 [cited 2017 Jul 18];18(4):657–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16223952

31. Patrick G. Murray -Manual of Clinical Microbiology. An Introd to Med

Chem [Internet]. 2013;413–62. Available from: https://bookshelf.vitalsource.com/#/books/9780191005862/

32. Luce E. Koneman's Color Atlas and Textbook of Diagnostic Microbiology, 6th Edition. Vol. 125, Plastic and reconstructive surgery. 2010. p. 414–5.

33. The Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing CLSI supplement M100S. Clinical and Laboratory Standards Institute, Wayne, PA. 2016. 256 p.

34. Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC ??-lactamases in Enterobacteriaceae lacking chromosomal AmpC ??-lactamases. J Clin Microbiol. 2005;43(7): 3110–3.

35. Akhter S. Prevalence and Detection of AmpC β -Lactamases in Gram Negative Bacilli from BIHS Hospital, Mirpur, Dhaka. Internaional Journl Res Stud Microbiol Biotechnol. 2015;1(2):1–6.

36. Samaha-kfoury JN, Araj GF. Clinical review spectrum lactamases. Bmj. 2003;327(22 November 2003):1209–13.

37. Mahon CR, Lehman DC, Manuselis G. Diagnostic Microbiology. Vol. 53, Library of congress cataloging in publication data. 2011. 1689-1699 p.

38. Johnson JR. Laboratory diagnosis of urinary tract infections in adult patients. Clin Infect Dis. 2004;39(6):873; author reply 873-874.

39. Scott B&. General Principles in Clinical.

40. Cheesbrough M. District laboratory practice in tropical countries. Vol. Part 1, District laboratory practice in tropical countries Part 2. 2006. (105 -115) p.

41. Roopa TJ, Sudha SS. Antimicrobial susceptibility of Extended Spectrum β -Lactamase (ESBL) producing Uropathogens isolated from ICU patients. 2010;1:23–31.

42. Deka N. Original Research Article Comparison of Tissue Culture plate method , Tube Method and Congo Red Agar Method for the detection of biofilm formation by Coagulase Negative Staphylococcus isolated from Non-clinical Isolates. IntJCurrMicrobiolAppSci. 2014;3(10):810–5.

43. Vinoth M, Prabagaravarthanan R, Bhaskar M. Prevalence of microorganisms causing catheter associated urinary tract infections (CAUTI) among catheterised patients admitted in a tertiary care hospital. 2017;5(6):2367–72.

44. Sayal P, Sandhu R, Singh K, Dev P. Slime production among uropathogenic bacterial isolates: Evaluating different phenotypic detection methods. Ann Pathol Lab Med [Internet]. 2017;4(1):0–5. Available from: http://www.pacificejournals.com/journal/index.php /apalm/ article/ view/apalm1064

45. Beyene G, Tsegaye W. Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in jimma university specialized hospital, southwest ethiopia. Ethiop J Health Sci [Internet].

2011;21(2):141–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22434993%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3275859.

46. Mangukiya JD, Patel KD, Vegad MM. Study of incidence and risk factors of urinary tract infection in catheterised patients admitted at tertiary care hospital. 2015;3(12):3808–11.

47. A.B.Mulhall. Bacteriuria during indwelling urethral-catheterization. J Hosp Infect. 1998;11:253–62.

48. Johnson JR1, Roberts PL, Olsen RJ, Moyer KA SW. Prevention of catheter-associated urinary tract infection with a silver oxide-coated urinary catheter: clinical and microbiologic correlates. J Infect Dis. 1990;162(5):1145–50.

49. Kulkarni SG, Talib SH, Naik M, Kale A. Profile of Urinary Tract Infection in Indwelling Catheterized Patients. IOSR J Dent Med Sci. 2014;13(4):132–8.

50. Nirmanmoh Bhatia SP. rinary atheterization in Medical Wards. J Glob Infect Dis. 2010;2(2):83–90.

51. Jayasukhbhai D. Mangukiya, Komal D. Patel MMV. Study of incidence and risk factors of urinary tract infection in catheterised patients admitted at tertiary care hospital. ijrms. 2015;3(12):2310–6312.

52. Felix K, Bellush M, Bor B. Guide to Preventing Urinary Tract Infections. APIC [Internet]. 2014;87. Available from:

http://apic.org/Resource_/EliminationGuideForm/0ff6ae59-0a3a-4640-97b5-eee38b8bed5b/File/CAUTI_06.pdf

53. Nicolle LE1. Catheter-related urinary tract infection. Pubmed. 2005;22(8):627–39.

54. Eshwarappa M1, Dosegowda R, Aprameya IV, Khan MW, Kumar PS KP. Clinico-microbiological profile of urinary tract infection in south India. indian J Nephrol. 2011;1(30):4065.

55. Niveditha S, Pramodhini S, Umadevi S, Kumar S, Stephen S. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). J Clin Diagnostic Res. 2012;6(9):1478–82.

56. Godaly G, Svanborg C. Urinary tract infections revisited. Kidney Int [Internet]. 2007;71(8):721–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17429419

57. Merle V, Germain JM, Bugel H, Nouvellon M, Lemeland JF, Czernichow P, et al. Nosocomial urinary tract infections in urologic patients: Assessment of a prospective surveillance program including 10,000 patients. Eur Urol. 2002;41(5):483–9.

58. Munoz-Davila M. Role of Old Antibiotics in the Era of Antibiotic Resistance. Highlighted Nitrofurantoin for the Treatment of Lower Urinary Tract Infections. Antibiotics [Internet]. 2014;3(1):39–48. Available from: http://www.mdpi.com/2079-6382/3/1/39/

59. Eisen DP. Urinary tract infections due to extended-spectrum beta-lactamase-producing Gram-negative bacteria: Identification of risk factors and outcome predictors in an Australian tertiary referral hospital. Int J Infect Dis [Internet]. 2015;34:79–83. Available from: http://dx.doi.org/10.1016/j.ijid.2015.03.006

60. Sujatha R and Pal. Antibiotic Resistance Pattern of The Hospital And Community Acquried Isolates Of Uropathogen In A Teritary Care Centre at Kanpur. Rama UnivJMed Sci 2015. 2015;1(1):10–7.

61. Sahm DF, Thornsberry C, Mayfield DC, Jones ME. Multidrug-resistant urinary tract isolates of *Escherichia coli*: prevalence and patient demographics in the United States in 2000. Antimicrob Agents Chemother. 2001;45(5):1402–6.

62. Aaftab GP. Multidrug Resistance Pattern in Confirmed Cases of Catheter Associated Urinary Tract Infection in a Tertiary Care Hospital : A Prospective Study. 2017;6(1):543–9.

63. Amine AEK, Helal MOM, Bakr WMK. Evaluation of an intervention program to prevent hospital-acquired catheter-associated urinary tract infections in an ICU in a rural Egypt hospital. GMS Hyg Infect Control [Internet]. 2014;9(2):Doc15. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4141633&tool=pmcentrez&rendertype=abstract

64. Tayebi Z, Seyedjavadi SS, Goudarzi M, Rahimi MK. Frequency and antibiotic resistance pattern in gram positive uropathogenes isolated from

hospitalized patients with urinary tract infection in Tehran , Iran Running title : Antibiotic resistance of Gram Positive uropathogenes. 2014;2014:1–9.

65. Goudarzi M, Seyedjavadi SS, Goudarzi H, Boromandi S, Ghazi M, Azad M, et al. Characterization of coagulase-negative staphylococci isolated from hospitalized patients in Tehran, Iran. J Paramed Sci. 2014;5(2).

66. Guanche-Garcell H, Requejo-Pino O, Rosenthal VD, Morales-Pérez C, Delgado-González O, Fernández-González D, et al. Device-associated infection rates in adult intensive care units of Cuban university hospitals: International Nosocomial Infection Control Consortium (INICC) findings. Int J Infect Dis [Internet]. 2011 May 1 [cited 2017 Aug 10];15(5):e357-62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21450505

67. Sheik GB, Sannat A, Al-rowis B. Prevalence , Risk Factors and Antimicrobial Susceptibility of Beta Lactamase Producing Enterobacteriacea in a Tertiary Care Centre of Saudi Arabia. 2016;5(2):98–109.

68. Amreliwala S, Durgad S, Poojary A. Original Research Article Carbapenem sparing options for the treatment of ESBL and AmpC producing Enterobacteriaceae in hemodynamically stable patients an in vitro study. 2015;4(2):513–21.

69. Oberoi L, Singh N, Sharma P, Aggarwal A. ESBL, MBL and Ampc β lactamases producing superbugs - Havoc in the intensive care units of

Punjab India. J Clin Diagnostic Res. 2013;7(1):70–3.

70. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol. 1985;22(6): 996–1006.

71. Ruchi A Tayal, Sujata M Baveja AS De. Analysis of biofilm formation and antibiotic susceptibility pattern of uropathogens in patients admitted in a tertiary care hospital in India. Int J Heal allied Sci. 2015;4(4): 247–52.

72. IqbalIV AHJUFKMOAKM. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis vol15 no4 Salvador July/Aug 2011. 2011;15(4):1413.

73. Id A, Author C. Biofilm Producing Vibrio Species Isolated from Siloso Beach , Singapore : A Preliminary Study Biofilm Producing Vibrio Species Isolated from Siloso Beach , Singapore : A Preliminary Study. 2012;1–6.

74. Ghanwate NA, Thakare P V, Bhise PR, Tayde S. Prevention of Biofilm Formation in Urinary Catheters by Treatment with Antibiofilm Agents. Int J. 2014;3(4):2–5.

75. WE S. Catheter-associated urinary tract infections: epidemiology, pathogenesis, and preventionle. Catheter Urin tract Infect Epidemiol Pathog Prev.

APPENDIX - I

ABBREVIATIONS

CAUTI	-	Catheter associated urinary tract infection
UTI	-	Urinary tract infection
CDC	-	Centers for Disease Control and Prevention
SUTI	-	Symptomatic urinary tract infection
CFU	-	Colony forming unit
TCP	-	Tissue culture plate method
TM	-	Tube method
CRM	-	Congo red method
CNF	-	Cytotoxic necrotising factor
USP	-	Uropathogenic strain specific proteins
LPS	-	Lipopolysaccharide
ESBL	-	Extended spectrum of betalactamase
MHA	-	Mueller-Hinton agar plate
MIC	-	Minimum Inhibitory Concentration
ATCC	-	American Type Culture Collection

KPC	-	Klebsiella producing carbapenamase
OPD	-	outpatient department
CLED	-	Cystine lactose electrolyte deficient agar
GPC	-	Gram positive cocci
GNB	-	Gram negative bacilli
CoNS	-	Coagulase negative Staphylococcus
CLSI	-	Clinical & Laboratory Standards Institute
DDST	-	Double disk synergy test
ELISA	-	Enzyme linked immunosorbant assay

APPENDIX - II

STAINS, REAGENTS AND MEDIA

PREPARATION OF GRAM STAIN

GRAM STAIN REAGENTS

1. Methyl violet - Primary stain

   Methyl violet 10 g

   95% ethyl alcohol 100 ml

   Distilled water 1 L

2. Gram's Iodine – Mordant

   Iodine 10 g

   Potassium iodide 20g

   Distilled water 1 L

3. Acetone - Decolouriser

4. Dilute Carbol Fuchsin - Counter stain

   Basic fuchsin 0.3g

   95% Ethyl alcohol 10 ml

   Phenol crystals, melted 5 ml

   Distilled water 95 ml

Basic fuchsin was dissolved in alcohol. 5 % phenol solution was added and was allowed to stand overnight. Then the solution filtered through coarse filter paper.

MEDIA USED:

Cystine lactose electrolyte deficient (CLED) medium

Ingredients

Peptone	4g
Tryptone	4g
Meat extract powder	3g
Lactose	10g
L-cystine	0.128g
Bromothymol blue	0.02g
Agar	15g
Water	1 litre

Preparation

Suspend the ingredients in the water, bring to boil to dissolve, sterilize at 121ºC for 15 min and mix well before pouring.

PREPARARION OF BLOOD AGAR

Nutrient agar 100 ml

Sheep blood (defibrinated) 10 ml

- sterile nutrient agar was melted by steaming and cooled to 45°C.
- 5% -10% sheep blood was added aseptically with constant shaking.
- blood was mixed with molten nutrient agar thoroughly but gently, to avoid froth formation.
- To remove the bubbles, media was flamed.
- immediately poured into petri dishes and allowed to set.

PREPARATION OF MUELLER – HINTON AGAR

Contents:

Beef extract 2.0 gm

Acidicase Peptone 17.5 gm

Starch 1.5 gm

Agar 17.0 gm

Distilled water 1000 ml

Final pH 7.4 + 0.2

Dissolve the ingredients in one litre of distilled water. Mix thoroughly. Heated with frequent agitation and boiled for one minute. Dispensed and sterilized by autoclaving at 121ºC for 15 minutes. Should not be over heated.

MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION:

1. **Catalase Test:**

   3% hydrogen peroxide.

2. **Oxidase Reagent:**

   Tetra methyl p-phenylene diamine dihyrochloride- 1% aqueous solution.

3. **Indole test:**

   Kovac's reagent

   Amyl or isoamyl alcohol 150ml

   Para dimethyl amino benzaldehyde 10g

   Concentrated hydrochloric acid 50ml

   Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4. **Simmon's Citrate Medium:**

   Koser's medium 1 litre

   Agar 20g

   Bromothymol blue 0.2% 40ml

   Dispense, autoclave at 121°C for 15 min and allow to set as slopes.

5. Triple Sugar Iron medium:

Beef extract 3g

Yeast extract 3g

Peptone 20g

Glucose 1g

Lactose 10g

Sucrose 10g

Ferric citrate 0.3g

Sodium chloride 5g

Sodum thiosulphate 0.3g

Agar 12g

Phenol red 0.2% solution 12ml

Distilled water 1 L

Heat to dissolve the solids, add the indicator solution, mix and tube.

Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

6. Mannitol motility medium

Agar 5g

Peptone 1g

Potassium nitrate 1g

Mannitol 2g

Phenol red indicator

Distilled water l000ml

pH 7.2

7. Nitrate medium:

Potassium nitrate 0.2g

Peptone 5g

Distilled water 1000ml

The above contents were mixed and tubed in 5 ml amounts and autoclaved at 121ºc for 15 minutes.

Test reagent: Solution A: 8 g of sulphanilic acid was dissolved in 1 L of acetic acid 5 mol/litre Solution B:5 g of alpha-naphthlamine in 1 L of acetic acid 5 mol/litre. Immediately before use, equal volumes of solutions A and B were mixed to get the test reagent.

8. Decarboxylase media:

Moeller decarboxylase broth base:

Peptone 5 g

Beef extract 5 g

Bromocresol purple 0.01 g

Cresol red 0.005 g

Glucose 0.5 g

Pyridoxal 0.005 g

Distilled water 1 litre

Final pH 6

Aminoacid:

Add 10g of the levo form of the aminoacid for 1000ml. Mix and dispense in sterile tubes.

9. Hugh & Leifson's Oxidation –Fermentation test:

Peptone 2g

Sodium chloride 5g

D-glucose 10g

Bromothymol blue 0.03g

Agar 3.0g

Dipotassium phosphate 0.30g

Distilled water 1litre

pH =7.1

Basal medium is autoclaved. 1% of sterile sugar solutions is added to the basal medium. Dispense into sterile test tubes without slant.

ANNEXURE – 1

INSTITUTIONAL ETHICAL COMMITTEE

CHENGALPATTU MEDICAL COLLEGE, CHENGALPATTU

Title of Work	:	A study on bacteriological profile and role of biofilm forming organisms in catheter associated urinary tract infection in a tertiary care hospital.
Principal Investigator	:	Dr.I.Gunaparvathy,
Designation	:	1st Year Post Graduate in Microbiology,
Co-Investigators	:	1. Dr.A.Vijayalakshmi,MD., Professor & Head Department of Microbiology 2.Dr.Ashok Thiyagarajan,Mch,. Professor & Head Department of Urology 3. Dr.A.V.Sowmya, MD,. Assistant Professor, Department of Microbiology
Department	:	Microbiology

The request for an approval From the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 29.02.2016 at the Medical Education Unit, Government Chengalpattu Medical College, Chengalpattu at 11.00 PM.

The Members of the committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal Investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of any changes in study procedure, site, investigator investigation or guide or any other changes.
2. You should not deviate from the area of work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions.
4. You should abide to the rules and regulations of the institution(s).
5. You should complete the work within the specific period and if any extension is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of work.

MEMBER SECRETARY,
IEC, CHENGALPATTU MEDICAL COLLEGE
CHENGALPATTU.

DEAN
CHENGALPATTU MEDICAL COLLEGE
CHENGALPATTU.

ANNEXURE – II

PROFORMA

Name : **IP no :**

Age : **Ward :**

Sex :

Occupation :

Address :

Presenting complaints:

Underlying illness:

Diabetes mellitus-

Tuberculosis-

Others

Prior antibiotic therapy :

Physical examinations :

Laboratory evaluation :

Sample collected : **Date:**

Microbiological investigation :

Macroscopic examination :

Microscopic examination :

Gram's stain :

Culture :

Biochemical reaction:

clinical isolate:

Biofilm formation:

Antimicrobial sensitivity pattern :

ANNEXURE – III

CONSENT FORM

(This is only a guideline –Relevant changes to be made as per the study requirements)

Title of the study : **"A STUDY ON BACTERIOLOGICAL PROFILE AND ROLE OF BIOFILM FORMING ORGANISMS IN CATHETER ASSOCIATED URINARY TRACT INFECTION IN A TERTIARY CARE HOSPITAL."**

Name of the participant :_____

Name of the Investigator : Dr.I.Gunaparvathy

Name of the Institution : Chengalpattu Medical College Hospital

Documentation of the informed consent.

I _____have read the information in this form (or it has been read to me).I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in **"A STUDY ON BACTERIOLOGICAL PROFILE AND ROLE OF BIOFILM FORMING ORGANISMS IN CATHETER ASSOCIATED URINARY TRACT INFECTION IN A TERTIARY CARE HOSPITAL."**

1. I have read and understand this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past_____including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past_____.
9. I have not donated blood within the past_____- Add if the study involves extensive blood sampling.
10. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.
11. I am also aware that the investigator may treatment my participated in the study at any time for any reason, without my consent.

12. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt.Agencies, and IEC. I understand that they are publicly presented.

13. I have understood that my identity will be kept confidential if my data are publicly presented.

14. I have had my questions answered to my satisfaction.

15. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the Investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature/thumb impression of the participant (or legal representative if participant incompetent)

Name_____signature_____Date_____

Name and signature of impartial witness (require for illiterate patients)

Name_____signature_____Date_____

Address and contact number of the impartial witness:

_____

Name and signature of the investigator or his representative obtaining consent:

Name_____signature_____Date_____

_____

Name and signature of the investigator or his representative obtaining consent:

Name_____signature_____Date_____

NOTE:-

For observational studies in nature or those in which only patient's tissue, body fluids are collected for any kind of analysis the following elements in the patient information leaflet will need be included – background of the study the purpose for which the sample will be used: confidentially of data are right to refuse to give specimens should be included.

Points 6, 7,8,9,10,11 of consent document may be excluded in such cases.

INFORMATION SHEET

We are conducting a study on **"A STUDY ON BACTERIOLOGICAL PROFILE AND ROLE OF BIOFILM FORMING ORGANISMS IN CATHETER ASSOCIATED URINARY TRACT INFECTION IN A TERTIARY CARE HOSPITAL."** in Chengalpattu Medical College Hospital, Chengalpattu.

For this your participation may be of immense value.

We are selecting patients who satisfy our inclusion criteria are included in the study.

The privacy of the patients will be maintained throughout the study, in the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Participation depends on patients own voluntary decision. Their decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator

Dr.I.Gunaparvathy

Signature of the Participant

Date :

Place :

ஆராய்ச்சிஒப்புதல்கடிதம்

ஆராய்ச்சிதலைப்பு: செங்கல்பட்டு அரசு பொது மருத்துவமனையில் சிறுநீரகவியல் துறையில் சிகிச்சை பெறும் நோயாளிகளின் சிறுநீர் வெளியேறும் செயற்கை குழாயில் வளரும் நுண்ணுயிர் கிருமிகள் மற்றும் அவை உற்பத்தி செய்யும் பையோபிலிம் தன்மையை பற்றிய ஆய்வு.

இடம்: **பெயர்**:

கையொப்பம்:

தேதி:

.

திரு/திருமதி _____

_____

என்றவிலாசத்தில்வசிக்கும்நான்,
எனக்குஅளிக்கப்பட்டதகவல்படிவத்தில்உள்ளவிஷயங்களைப்படித்தும்கேட்டும்புரிந்து கொண்டேன்.

இந்தஆய்விற்குத்தேவையான சிறுநீர் மற்றும் சிறுநீர் **வெளியேறும்** செயற்கை குழாய் தருவதற்கு நான் சம்மதிக்கிறேன்.

இந்தஆய்வில்பிறரின்நிர்ப்பந்தமின்றிஎன்சொந்தவிருப்பத்தின்பேரில்நான்பங்கு பெறுகிறேன்.

ஆய்வில்தொடர்ந்துபங்குபெறவிருப்பம்இல்லைஎன்றால்விலகிக்கொள்ளலாம் என்றும்அறிந்துகொண்டேன்.

ஆய்வின்முடிவினைசொந்தஅடையாளங்களைவெளியிடாமல்மருத்துவ ஆராய்ச்சிக்காக பயன்படுத்திக்கொள்ள சம்மதிக்கிறேன்.

நாள்:

இடம்: கையொப்பம்

தகவல்படிவம்

ஆராய்ச்சிதலைப்பு: செங்கல்பட்டு அரசு பொது மருத்துவமனையில் சிறுநீரகவியல் சிகிச்சை பெறும் நோயாளிகளின் சிறுநீர் வெளியேறும் செயற்கை குழாயில் வளரும் நுண்ணுயிர் கிருமிகள் மற்றும் அவை உற்பத்தி செய்யும் பையோபிலிம் தன்மை பற்றிய ஆய்வு.

- இந்த ஆய்வு மருத்துவர் இ.குணபார்வதி அவர்கலால் அனுபவம்

  வாய்ந்த மருத்துவர்களின் உதவியோடு நடத்தப்படுகிறது.

- இந்த ஆய்வின்போது என்னுடைய சிறுநீர் மற்றும் சிறுநீர் வெளியேறும் செயற்கை குழாய் பரிசோதனை எடுக்கும் போது எந்த விதமான பக்கவிளைவுகளும் ஏற்படாது என்று நான் ஒப்புக்கொள்கிறேன்.

- இந்த ஆய்வின்தொடக்கத்திலும்முடிவிலும் சிறுநீரகம் மற்றும் சிறுநீரகம் வெளியேறும் பரிசோதனை கண்டறியும் சோதனைகள்செய்யப்படும்.

- நோயின் தன்மைகளை வெளியிடும்போது தங்களது பெயரையோ,

  அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக்

  கொள்கிறோம்.

- இந்த ஆய்வில் பங்கேற்பது உங்களுடைய விருப்பத்தின் பேரில்தான் இருக்கிறது. மேலும் நீங்கள் எந்நேரமும் இந்த ஆய்விலிருந்து பின்வாங்கலாம் என்பதையும்

  தெரிவித்துக் கொள்கிறோம்.

- இந்த சிறப்புபரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின்போது அல்லது

  ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

நாள்:

இடம்: கையொப்பம்

ANNEXURE – IV

MASTER CHART

S.no	OP.NO	Age	Sex	Diagnosis	Fever	Abdomen pain	loin pain	DM	HT	Others	Gram Stain	Culture	Tube Method	Congo Red	Tissue Culture	Cefoxitin	Amikacin	Ampicilin	Gentamycin	Norfloxacin	Ciproflixacin	Ofloxacin	Cotrimoxazole	Cefotaxine	Ceftriaxone	Ceftazidime	CFCV	CEV	Nitrofurontion	Azteronam	CFS	Imipenam	Meropenam	PTZ	Tetracycline	Cephelexin	AC	Va	CPM	ESBL	AMPC	MR
1	1244	80	M	BPH	A	P	P	-	-	-	+	K.pneumoniae	W	N	M	S	R	R	R	R	R	R	R	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S			
2	166	32	M	Urethral stricture	A	P	P	-	-	-	+	P.aeruginosa	S	N	S	S	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	S	R		S			
3	1501	75	M	TURP	A	P	A	-	-	-	+	K.pneumoniae	N	N	N	S	R	R	R	R	R	R	R	S	S	S	S	S	R	S	S	S	S	S	S	R	S		S			
4	1795	60	M	TRUP	P	P	A	-	-	-	+	K.oxytoca	N	N	N	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		R	+		
5	1289	70	M	BPH	A	P	A	-	-	HbSAg+	-	NG																														
6	83	70	M	CALCULUS	A	P	P	-	-	-	+	Pr.mirabilis	S	N	S	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		R	+		
7	457	90	M	BPH	A	P	A	+	-	-	-	NG																														
8	1731	80	M	BPH	A	P	A	-	-	-	-	NG																														
9	92	80	M	BPH	A	P	P	-	-	-	+	CONS	S	N	S	R	R	R	R	R	R	S	R	R	R	R	S	S	S	S	S	S	S	S	R	R	S	S	S			+
10	57	65	M	BPH	A	P	A	-	-	-	-	NG																														
11	1254	70	F	CARCINOMA VULVA	A	P	A	-	-	-	+	Pr.mirabilis	W	N	N	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S		S			
12	183	75	M	BPH	P	P	A	-	-	-	+	CONS	M	N	M	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S			+
13	114	60	M	BPH	A	P	A	-	+	-	+	E.coli	S	N	S	S	S	R	R	S	S	R	R	S	S	S	S	S	S	R	S	S	S	S	S	S	R		S			
14	849	60	M	BPH	A	P	A	+	-	-	+	NG																														
15	345	55	M	CYSTITIS	A	P	P	+	-	-	+	S.aureus	M	N	M	S	R	R	R	R	R	S	R	R	R	S	S	S	S		S	S	S	S	R	S	S	S	S			
16	820	60	M	BPH	A	P	P	+	-	-	+	S.aureus	W	N	M	R	S	R	R	S	R	S	R	S	S	S	S	S	S		S	S	S	S	R	S	S	S	S			+
17	1032	55	M	CARCINOMA CERVIX	A	P	A	-	-	-	+	Enterococci	N	N	N	S	S	S	R	R	S	S	R	S	S	S	S	S	R		S	S	S	S	S	S	S	S	S			
18	271	77	M	BPH	A	P	A	-	-	-	-	NG																														
19	187	77	M	BPH	A	P	A	-	-	-	+	Candida																														
20	1236	67	M	BPH	P	P	A	+	-	-	+	E.coli	M	N	S	S	R	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R		S			
21	462	70	M	BPH	A	P	A	+	-	-	+	S.aureus	N	N	N	R	R	R	R	R	R	S	R	S	S	S	S	S	S		S	S	S	S	S	R	R	S	S			+
22	835	70	M	BPH	A	P	A	+	+	-	-	NG																														
23	1085	80	M	BPH	A	P	A	+	-	-	+	K.pneumoniae	M	N	M	R	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S		R		+	
24	108	60	M	BPH	A	P	A	+	-	-	+	S.aureus	M	N	S	R	R	R	R	R	R	S	R	R	R	R	S	S	S		R	S	S	S	R	R	R	S	S			+
25	284	63	M	BPH	A	P	A	-	-	-	+	CONS	S	N	S	S	S	S	R	S	S	R	R	R	R	R	S	S	S		S	S	S	S	R	S	R		S			
26	1294	80	M	BPH	A	P	A	-	-	-	-	NG																														
27	1475	35	M	BPH	A	P	P	-	-	-	+	K.pneumoniae	N	N	N	R	S	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	S	S	R	S	S		S		+	
28	893	55	M	BPH	A	P	P	-	-	-	+	Pr.vulgaris	N	N	N	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	R	S	R		R	+		
29	380	50	M	CARCINOMA	A	P	P	+	-	-	+	P.auregnosa	S	M	S	S	S	R	R	R	R	R	R	S	S	S	S	S	R	S	R	S	S	S	R	R	R		R			
30	170	35	M	BPH	A	P	A	-	-	-	+	Candida																														
31	2049	45	M	STICTURE	A	P	A	-	-	-	+	E.coli	S	N	S	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	S	S	S	R	R	R		S		+	
32	1349	60	M	BPH	A	P	A	-	-	-	+	S.aureus	M	N	M	R	S	R	R	R	R	S	R	S	S	S	S	S	S		S	S	S	S	R	R	R	S	S			+
33	1349	65	M	BPH	P	P	A	+	-	-	+	Enterococci	N	N	N	R	R	R	R	R	S	S	S	R	R	R	S	S	R		S	S	S	S	S	R	S		S			
34	472	22	F	Urethral stricture	A	P	P	+	-	-	+	S.aureus	N	N	N	S	R	S	R	R	R	S	S	S	S	S	S	S	S		S	S	S	S	S	R	S	S	R			
35	2371	75	M	BPH	A	P	A	-	-	-	+	E.coli	N	N	N	S	S	R	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S	S	S	R		S	+		

S.no	OP.NO	Age	Sex	Diagnosis	Fever	Abdomen pain	loin pain	DM	HT	Others	Gram Stain	Culture	Tube Method	Congo Red	Tissue Culture	Cefoxitin	Amikacin	Ampicilin	Gentamycin	Norfloxacin	Ciprofilaxacin	Ofloxacin	Cotrimoxazole	Cefotaxine	Ceftriaxone	Ceftazidime	CFCV	CEV	Nitrofurontion	Azteronam	CFS	Imipenam	Meropenam	PTZ	Tetracycline	Cephelexin	AC	Va	CPM	ESBL	AMPC	MR
36	1284	77	M	BPH	A	P	A	+	-	-	+	Pr.mirabilis	N	N	N	R	S	R	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		R	+		
37	1821	80	M	BPH	A	P	A	-	-	-	+	E.coli	S	M	S	S	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
38	731	43	M	CALCULUS	A	P	A	-	-	-	+	CONS	M	N	M	R	R	R	R	R	R	S	R	R	R	R	S	S	S		S	S	S	S	S	R	S	S	S			+
39	1795	65	M	BPH	A	P	A	-	+	-	-	NG																														
40	169	65	M	BPH	A	P	A	+	-	-	+	E.coli	M	N	S	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
41	1150	74	M	BPH	A	P	A	-	-	-	-	NG																														
42	1035	70	M	BPH	A	P	A	+	-	-	+	E.coli	S	M	M	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
43	72	60	M	BPH	A	P	A	-	-		-	NG																														
44	1584	50	M	BPH	A	P	P	-	-		-	E.coli	M	N	M	S	S	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
45	331	75	M	BPH	A	P	P	-	-	-	+	P.auregnosa	S	N	S	S	R	R	R	R	R	R	R	R	S	S	S	S	S	R	S	S	S	S	R	R	R		S			
46	732	55	M	BPH	A	P	A	-	-	-	-	NG																														
47	935	60	M	BPH	A	P	A	+	-	-	+	Pr.vulgaris	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	S	R	R	S		S			
48	735	55	M	BPH	A	P	A	-	-	-	-	NG																														
49	756	80	M	BPH	A	P	A	-	-	-	-	NG																														
50	759	70	M	BPH	A	P	A	+	-	-	+	E.coli	S	S	S	S	S	R	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S	R	R	S		R	+		
51	792	60	M	Urethral stricture	A	P	A	+	-	-	+	E.coli	N	N	N	R	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R		S		+	
52	813	43	F	CALCULUS	A	P	A	-	-	-	-	NG																														
53	316	70	M	BPH	A	P	A	-	-	-	-	NG																														
54	820	66	M	BPH	A	P	P	-	-	-	+	E.coli	M	N	S	R	S	R	R	R	R	S	R	S	S	S	S	S	S	R	S	S	S	S	S	R	S		R		+	
55	852	55	F	Urethral stricture	A	P	A	-	-	-	-	NG																														
56	1857	75	M	BPH	A	P	A	-	-	-	+	P.auregnosa	S	M	S	S	R	R	R	R	R	R	R	S	S	S	S	S	S	R	R	S	S	S	S	R	R		S			
57	858	75	M	BPH	A	P	A	-	-	-	-	NG																														
58	861	63	M	BPH	A	P	A	+	-	-	+	Pr.mirabilis	N	N	N	S	S	R	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
59	863	58	M	BPH	A	P	A	+	-	-	+	E.coli	M	N	M	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
60	870	60	M	BPH	A	P	A	-	+	-	-	S.aureus	S	M	S	S	S	S	R	S	R	S	R	R	R	R	S	S	S		S	S	S	S	S	S	S	S	S			
61	324	53	F	Urethral stricture	A	P	A	-	+	-	-	NG																														
62	1689	70	M	BPH	A	P	A	+	-	-	+	E.coli	M	N	M	S	R	R	R	R	S	S	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
63	900	65	M	BPH	A	P	A	+	-	-	-	NG																														
64	869	70	M	CARCINOMA PENIS	P	P	A	-	-	-	+	CONS	S	N	S	R	R	R	R	R	R	R	S	S	S	S	S	S	S		S	S	S	S	R	R	R	S	R				+
65	540	70	M	BPH	A	P	A	-	-	TB	+	NG																														
66	893	83	M	BPH	A	P	A	-	-		+	E.coli	N	N	N	S	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R		S			
67	894	65	M	BPH	A	P	A	-	-		-	NG																														
68	686	67	M	BPH	A	P	A	+	-		+	CONS	M	N	M	R	R	R	R	R	R	R	R	R	R	R	S	S	S		S	S	S	S	S	R	R	S	S				+
69	440	85	M	BPH	A	P	A	-	-		-	NG																														
70	1131	70	M	BPH	A	P	P	-	-	-	+	p.aeruginosa	M	M	S	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R		R			
71	1783	59	F	CARCINOMA VULVA	A	P	A	-	-	-	-	NG																														

S.no	OP.NO	Age	Sex	Diagnosis	Fever	Abdomen pain	loin pain	DM	HT	Others	Gram Stain	Culture	Tube Method	Congo Red	Tissue Culture	Cefoxitin	Amikacin	Ampicilin	Gentamycin	Norfloxacin	Ciprofilxacin	Ofloxacin	Cotrimoxazole	Cefotaxine	Ceftriaxone	Ceftazidime	CFCV	CEV	Nitrofurontion	Azteronam	CFS	Imipenam	Meropenam	PTZ	Tetracycline	Cephelexin	AC	Va	CPM	ESBL	AMPC	MR
72	982	24	F	CALCULUS	P	P	A	-	-		+	E.coli	N	N	N	S	S	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
73	2357	70	M	BPH	A	P	A	+	-		-	NG																													
74	984	92	M	BPH	A	P	A	-	-		+	E.coli	N	N	M	S	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
75	1085	80	M	BPH	A	P	A	-	-	-	+	E.coli	M	N	M	S	S	R	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S	R	R		S	+		
76	2514	60	M	BPH	A	P	A	-	-	-	-	NG																													
77	1017	60	M	BPH	A	P	P	+	-		+	p.aeruginosa	M	N	S	S	S	R	R	R	R	R	R	R	R	R	S	S	R	S	S	S	S	S	S	R	R		S			
78	2018	65	M	BPH	A	P	A	-	+		-	NG																													
79	1331	70	M	BPH	A	P	A	-	-	-	+	Pr.penneri	N	N	N	S	S	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	R	S	R	R		R			
80	352	70	M	BPH	A	A	P	-	-		-	NG																													
81	569	70	M	BPH	A	P	A	-	-		+	K.pneumoniae	M	N	S	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	R	S	R	S		S	+		
82	977	77	M	BPH	A	P	A	+	-		+	E.coli	M	N	M	S	S	R	R	R	R	R	R	R	R	S	S	S	S	R	S	S	S	S	S	R	S		S	+		
83	1483	70	M	BPH	A	P	A	-	-		-	NG																													
84	1453	70	F	Urethral stricture	A	P	A	-	-		-	E.coli	M	N	N	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
85	1685	65	M	BPH	A	P	A	-	-		+	K.oxytoca	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S		S			
86	881	60	M	BPH	A	P	A	-	-		+	terococccus feca	M	N	M	R	R	R	R	R	R	R	R	R	R	R	S	S	R		S	S	S	S	S	R	S	S	R			
87	925	35	M	BPH	A	P	A	-	-		-	NG																													
88	910	81	M	BPH	A	P	A	-	-	-	+	Pr.mirabilis	N	N	N	S	S	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R		S			
89	820	73	M	BPH	A	P	A	+	+	-	-	NG																													
90	720	72	M	CARCINOMA LUNG	A	P	P	+	-	-	+	E.coli	M	N	M	S	S	R	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
91	1856	78	M	BPH	A	P	A	-	-	-	-	NG																													
92	1314	75	M	BPH	A	P	A	-	-		+	E.coli	S	S	S	S	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	S		S	+		
93	567	55	M	BPH	A	P	A	-	-		+	Pr.mirabilis	M	N	M	R	S	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S		S			
94	1783	78	M	BPH	A	P	A	-	-		+	E.coli	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S		R			
95	1643	60	M	BPH	A	P	A	-	-		-	NG																													
96	830	80	M	BPH	A	P	P	+	-	-	+	S.aureus	M	N	M	R	S	R	R	R	R	R	R	S	S	S	S	S	S		S	S	S	S	S	R	S	S	S			+
97	567	56	F	Urethral stricture	A	P	A	-	+	-	-	NG																													
98	876	60	M	BPH	A	P	A	-	-	-	+	S.aureus	N	N	N	S	S	S	R	S	R	S	R	S	S	S	S	S	R		S	S	S	S	R	R	R	S	R			
99	890	60	M	BPH	A	P	A	-	-		-	E.coli	M	M	M	S	R	R	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S	S	R	S		S	+		
100	892	65	M	BPH	A	P	A	-	-		-	NG																													
101	879	72	M	CARCINOMA STOMACH	A	P	A	+	-	-	+	E.coli	S	N	S	S	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	S	S	S	R	R	S		R	+		
102	883	75	M	BPH	P	P	A	+	-	-	+	E.coli	N	N	N	S	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R		S			
103	886	60	M	BPH	A	P	A	-	+		+	E.coli	S	N	S	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		R	+		
104	256	70	M	BPH	A	P	A	-	-		-	NG																													
105	994	64	M	BPH	A	P	P	+	-	-	+	S.aureus	M	M	M	R	S	R	R	R	R	R	R	R	R	R	S	S	S		S	S	S	S	S	R	S	S	S			+
106	341	55	F	CARCINOMA CERVIX	A	P	A	+	-		-	NG																													
107	344	60	M	BPH	A	P	A	-	-		+	E.coli	M	N	M	S	S	R	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S		R			

S.no	OP.NO	Age	Sex	Diagnosis	Fever	Abdomen pain	loin pain	DM	HT	Others	Gram Stain	Culture	Tube Method	Congo Red	Tissue Culture	Cefoxitin	Amikacin	Ampicilin	Gentamycin	Norfloxacin	Ciprofilixacin	Ofloxacin	Cotrimoxazole	Cefotaxine	Ceftriaxone	Ceftazidime	CFCV	CEV	Nitrofurontion	Azteronam	CFS	Imipenam	Meropenam	PTZ	Tetracycline	Cephelexin	AC	Va	CPM	ESBL	AMPC	MR
108	1038	55	F	Urethral stricture	A	P	A	-	-		-	N G																														
109	1060	60	M	BPH	A	P	A	-	-		+	CONS	M	N	M	S	S	S	R	R	S	S	R	R	R	R	S	S	S		S	S	S	S	S	S	R	S	R			
110	1065	60	M	BPH	A	P	A	-	-		-	NG																														
111	1085	66	M	BPH	A	P	A	-	-		-	NG																														
112	2081	70	M	BPH	A	P	A	+	-		+	p.aeruginosa	S	M	S	S	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	R	R		S			
113	1060	60	M	BPH	A	P	A	+	-		+	K.pneumoniae	N	N	S	S	R	S	R	S	R	R	R	S	S	S	S	S	S	R	S	S	S	S	S	R	S		R			
114	1679	60	M	BPH	A	P	A	+	-		+	S.aureus	S	N	S	S	S	S	R	S	R	S	R	R	S	S	S	S	S		S	S	S	S	S	S	S	S	S			
115	1085	63	M	BPH	A	P	A	-	-		-	NG																														
116	1314	75	M	BPH	A	P	A	-	-		+	terococcus feaca	S	M	S	S	R	S	R	R	R	S	R	R	R	R	S	S	R		S	S	S	S	S	R	R	S	S			
117	362	60	M	BPH	A	P	A	-	-		-	NG																														
118	1274	62	M	BPH	A	P	A	-	-		+	S.aureus	M	M	M	S	R	S	R	R	R	R	R	R	R	R	S	S	S		S	S	S	S	S	R	R	S	R			
119	1873	57	M	BPH	P	P	A	-	-		+	P.auregnosa	M	N	M	S	R	R	R	R	R	R	R	R	R	R	S	S	R	S	S	S	S	S	S	R	R		S			
120	935	35	F	Urethral stricture	A	P	P	-	-		+	S.aureus	M	N	M	R	S	R	R	R	R	R	R	R	R	R	S	S	S		S	S	S	S	S	R	S	S	R			+
121	1504	60	M	BPH	A	P	A	-	-		-	NG																														
122	1203	47	M	BPH	A	P	A	-	-		+	S.aureus	N	N	N	S	S	S	R	R	R	R	R	S	S	S	S	S	S		S	S	S	S	S	R	S	S	R			
123	2594	60	M	BPH	A	P	A	+	-		-	NG																														
124	83	70	M	BPH	A	P	A	-			+	Pr.mirabilis	N	N	N	S	S	R	R	S	R	R	R	S	S	S	S	S	S	R	S	S	S	S	S	R	S		S			
125	1544	34	M	BPH	A	P	A	-	-		+	E.coli	S	M	S	R	S	R	R	R	S	S	S	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
126	567	37	M	BPH	A	P	P	-	-		+	K.oxytoca	N	N	N	R	S	R	R	R	R	S	R	S	S	S	S	S	S	R	S	S	S	S	S	R	R		S		+	
127	2283	30	M	BPH	A	P	A	-			+	E.coli	N	N	M	R	S	R	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		R	+		
128	1274	64	M	BPH	A	P	A	+	-		+	E.coli	M	N	S	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S	S	S	S	R	S		R	+		
129	1432	74	M	BPH	A	P	A	-			+	E.coli	M	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		S	+		
130	1335	35	M	BPH	A	P	P	-	-		-	NG																														
131	558	45	M	BPH	A	P	A	-	-		+	terococcus feaca	S	N	S	R	S	R	R	R	R	R	R	R	R	R	S	S	R		S	S	S	S	S	R	S	S	R			
132	1132	58	M	STICTURE	A	P	A	+	+		+	E.coli	M	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		R	+		
133	565	43	M	BPH	P	P	A	-	-		+	CONS	M	M	M	R	R	R	R	R	R	R	R	R	R	R	S	S	S		S	S	S	S	S	R	R	S	S			+
134	1419	53	F	Urethral stricture	A	P	A	+	-		+	E.coli	N	N	N	S	S	R	R	S	R	S	S	S	S	S	S	S	S		S	S	S	S	S	R	S		S			
135	578	34	M	BPH	A	P	P	-	-		+	K.pneumoniae	N	N	N	S	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
136	597	30	M	BPH	A	P	A	-	-		+	CANDIDA																														
137	1485	59	F	Urethral stricture	A	P	A	-	-		-	NG																														
138	612	35	M	CALCULUS	A	P	A	-	-		+	E.coli	S	M	S	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		R	+		
139	569	65	F	Urethral stricture	A	P	A	-	-		+	E.coli	M	N	S	S	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S		S			
140	649	32	M	BPH	A	P	A	-	-		-	NG																														
141	641	65	M	BPH	A	P	A	+	-		+	E.coli	M	M	M	S	S	R	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	R	R	R		S			
142	1783	50	F	CARCINOMA CERVIX	A	P	A	-	-		+	E.coli	M	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	R	R	R		S	+		
143	1534	58	M	BPH	A	P	A	-	-		-	NG																														

S.no	OP.NO	Age	Sex	Diagnosis	Fever	Abdomen pain	loin pain	DM	HT	Others	Gram Stain	Culture	Tube Method	Congo Red	Tissue Culture	Cefoxitin	Amikacin	Ampicilin	Gentamycin	Norfloxacin	Ciprofilxacin	Ofloxacin	Cotrimoxazole	Cefotaxine	Ceftriaxone	Ceftazidime	CFCV	CEV	Nitrofurontion	Azteronam	CFS	Imipenam	Meropenam	PTZ	Tetracycline	Cephelexin	AC	Va	CPM	ESBL	AMPC	MR
144	700	50	M	BPH	A	P	A	-	-		+	K.pneumoniae	N	N	N	S	S	R	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
145	1604	33	M	CALCULUS	A	P	A	-	-		+	E.coli	S	M	S	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	R	S	S	S	R	R	R		S	+		
146	1732	60	M	BPH	A	P	A	-	-		+	CANDIDA																														
147	1531	74	M	BPH	A	P	A	+	-		+	terococcus feaca	N	N	N	R	R	R	R	R	R	S	R	R	S	R	S	S	R		S	S	S	S	S	R	S	S	R			
148	742	30	M	Urethral stricture	A	P	A	-	-		-	NG																														
149	705	32	M	CALCULUS	P	P	A	-	+		+	Pr.mirabilis	M	M	M	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
150	763	30	M	Urethral stricture	A	P	P	-	-		+	E.coli	S	N	S	R	S	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	S	S	R	R		S		+	
151	1184	70	F	Urethral stricture	A	P	A	+	-		+	CONS	N	N	N	S	S	S	R	R	R	S	S	S	S	S	S	S	S		S	S	S	S	S	R	R	S	S			
152	1897	56	M	BPH	A	P	A	+	-		+	E.coli	M	N	S	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		R	+		
153	820	68	M	BPH	A	P	A	-	+		+	CANDIDA																														
154	833	54	M	BPH	A	P	A	-	-		+	E.coli	N	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		S	+		
155	825	76	M	BPH	A	P	A	-	-		-	NG																														
156	1965	83	M	BPH	A	P	P	-	-		+	P.auregnosa	M	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	R	S	R	S	S	S	R	R	S		R			
157	848	46	M	Urethral stricture	A	P	A	-	-		+	E.coli	N	N	N	S	S	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
158	875	52	M	BPH	A	P	A	-	-		+	CANDIDA																														
159	878	40	F	Urethral stricture	A	P	A	-	-		+	E.coli	M	N	M	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
160	956	43	F	Urethral stricture	A	P	A	-	+		+	K.pneumoniae	N	N	M	R	S	S	S	R	R	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	R		S		+	
161	1409	43	M	BPH	A	P	A	+	-		+	E.coli	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
162	2084	50	M	BPH	A	P	A	-	-		+	NG																														
163	1879	37	F	Urethral stricture	A	P	A	-	-		-	CANDIDA																														
164	958	75	M	BPH	P	P	A	-	-		+	E.coli	S	N	S	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		R	+		
165	1136	65	F	CARCINOMA VULVA	A	P	A	-	-		-	NG																														
166	1595	75	M	BPH	A	P	A	+	-		+	K.pneumoniae	M	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	R	S	S	S	S	R	R		S	+		
167	1243	40	M	Urethral stricture	A	P	A	-	-		-	NG																														
168	748	70	M	BPH	A	P	A	-	-		+	C.koseri	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
169	1356	54	M	BPH	A	P	A	-	-		+	E.coli	M	M	S	R	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
170	1456	25	F	Urethral stricture	A	P	A	-	-		-	NG																														
171	1437	45	M	BPH	A	P	P	-	-		+	E.coli	S	S	S	S	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		R	+		
172	345	49	M	BPH	A	P	P	-	+		+	E.coli	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
173	1384	55	F	CALCULUS	A	P	A	-	-		+	E.coli	N	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
174	475	38	F	Urethral stricture	A	P	A	-	-		-	NG																														
175	905	55	M	BPH	A	P	A	-	-		+	E.coli	M	M	M	S	S	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S		R			
176	456	64	M	BPH	P	P	A	+	-		+	K.oxytoca	M	M	M	S	S	R	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S	R	R	R		S	+		
177	2356	36	M	Urethralstricture	A	P	A	+	-		+	K.pneumoniae	N	M	M	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
178	678	50	M	BPH	A	P	A	-	+		+	E.coli	S	N	S	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	R	S	R	R	R		S	+		
179	436	60	M	BPH	A	P	A	-	-		+	E.coli	M	N	S	S	S	R	R	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S		R			

S.no	OP.NO	Age	Sex	Diagnosis	Fever	Abdomen pain	loin pain	DM	HT	Others	Gram Stain	Culture	Tube Method	Congo Red	Tissue Culture	Cefoxitin	Amikacin	Ampicilin	Gentamycin	Norfloxacin	Ciprofilixacin	Ofloxacin	Cotrimoxazole	Cefotaxine	Ceftriaxone	Ceftazidime	CFCV	CEV	Nitrofurontion	Azteronam	CFS	Imipenam	Meropenam	PTZ	Tetracycline	Cephelexin	AC	Va	CPM	ESBL	AMPC	MR
180	1264	32	M	Urethral stricture	A	P	A	-	-		-	NG																														
181	1323	42	M	BPH	A	P	A	-	-		+	P.auregnosa	N	N	M	S	S	R	R	R	R	R	R	R	R	S	S	R	S	R	S	S	S	R	R	R		R			
182	1543	36	M	BPH	A	P	A	-	-		-	NG																														
183	776	45	M	BPH	A	P	A	+	-		+	E.coli	N	N	N	S	S	R	R	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R		S			
184	1253	69	M	BPH	P	P	P	-	-		+	P.auregnosa	N	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	R	S	S	S	S	S	R	R		S			
185	467	45	M	CALCULUS	A	P	A	+	-		-	NG																														
186	576	67	M	BPH	A	P	A	-	-		+	E.coli	S	N	M	S	S	R	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
187	1198	55	F	Urethral stricture	A	P	A	-	-		+	Pr.mirabilis	N	N	N	S	S	R	S	R	R	S	R	R	R	S	S	S	S	S	S	S	S	S	R	S		S			
188	2789	37	M	BPH	P	P	A	-	-		-	NG																														
189	1674	65	M	BPH	A	P	A	+	-		+	E.coli	N	N	M	S	S	S	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	R	R		R	+		
190	1190	80	M	BPH	A	P	A	-	-		-	NG																														
191	1134	57	M	BPH	A	P	A	+	-		+	CANDIDA																														
192	1437	46	M	BPH	P	P	A	-	-		+	E.coli	N	M	M	S	S	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	S		S	+		
193	2345	37	F	Urethral stricture	A	P	A	-	-		+	E.coli	N	N	M	S	S	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	R	R	S		S			
194	3754	70	M	Urethral stricture	A	P	P	-	+		-	NG																														
195	1125	57	M	BPH	P	P	A	-	-		-	NG																														
196	987	65	M	BPH	A	P	A	-	-		+	P.auregnosa	M	S	S	S	S	R	R	R	R	R	R	S	S	S	S	S	R	S	R	S	S	S	R	R	R		S			
197	345	78	M	BPH	A	P	A	-	-		+	E.coli	M	M	M	S	S	R	R	R	R	R	R	S	S	R	S	S	S	R	S	S	S	S	S	R	S		R			
198	1183	65	M	BPH	A	P	A	+	-		+	terococcus feaca	M	M	S	S	R	S	R	R	R	S	R	R	R	R	S	S	S		S	S	S	S	S	R	S	S	S			
199	3245	75	M	BPH	A	P	A	-	-		+	CONS	M	N	M	S	S	S	S	R	R	R	R	R	R	R	S	S	S		S	S	S	S	S	R	R	S	S			
200	3325	54	M	Urethral stricture	A	P	A	-	+		-	NG																														
201	1123	45	M	BPH	A	P	A	-	-		+	E.coli	N	N	M	R	S	R	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	S	S	S	R	S		S		+	
202	1654	63	M	BPH	A	P	P	+	-		+	E.coli	N	N	S	S	S	R	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S	R	R	R		S	+		
203	835	65	M	BPH	A	P	P	-	-		+	Pr.mirabilis	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
204	1784	60	F	Urethral stricture	P	P	A	-	-		+	K.pneumoniae	N	N	N	S	R	R	R	R	R	S	R	R	R	R	S	S	R	R	R	S	S	S	S	R	R		S	+		
205	1589	75	F	Urethral stricture	A	P	A	-	-		+	E.coli	N	N	N	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		R	+		
206	1244	73	M	BPH	A	P	A	+	-		+	Pr.vulgaris	N	N	M	S	S	R	S	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
207	1332	58	M	BPH	A	P	A	-	-		+	K.pneumoniae	N	M	M	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		S	+		
208	1156	56	M	BPH	A	P	A	-	-		+	K.pneumoniae	N	N	N	R	S	R	R	R	R	R	R	S	S	S	S	S	R	R	S	S	S	S	S	R	S		S		+	
209	1186	59	M	BPH	A	P	A	-	-		+	E.coli	M	N	M	S	R	S	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		R	+		
210	874	45	M	BPH	A	P	A	-	-		-	NG																														
211	1100	70	M	BPH	A	P	A	-	-		+	K.pneumoniae	M	N	M	S	S	S	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S	S	R	S		R	+		
212	734	67	M	CARCINOMA LUNG	P	P	P	-	-		+	P.auregnosa	M	M	M	S	S	R	R	R	R	R	S	S	S	S	S	S	R	S	S	S	S	S	S	R	R		S			
213	275	46	F	Urethral stricture	A	P	A	-	-		+	Pr.mirabilis	N	N	N	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
214	489	48	M	BPH	A	P	A	-	-		+	E.coli	N	N	N	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
215	2017	35	M	BPH	A	P	A	-	-		-	NG																														

S.no	OP.NO	Age	Sex	Diagnosis	Fever	Abdomen pain	loin pain	DM	HT	Others	Gram Stain	Culture	Tube Method	Congo Red	Tissue Culture	Cefoxitin	Amikacin	Ampicilin	Gentamycin	Norfloxacin	Ciprofilixacin	Ofloxacin	Cotrimoxazole	Cefotaxine	Ceftriaxone	Ceftazidime	CFCV	CEV	Nitrofurontion	Azteronam	CFS	Imipenam	Meropenam	PTZ	Tetracycline	Cephelexin	AC	Va	CPM	ESBL	AMPC	MR
216	2036	26	F	Urethral stricture	A	P	A	-	-		+	K.pneumoniae	N	N	N	R	S	S	R	R	R	R	S	S	S	S	S	S	R	R	S	S	S	S	S	R	R		S		+	
217	2356	56	M	BPH	A	P	A	-			-	NG																														
218	1147	67	M	BPH	A	P	A	+			-	NG																														
219	1155	56	M	BPH	A	P	P	-	-		+	E.coli	M	M	M	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	S		R	+		
220	2965	48	M	BPH	A	P	P	-	-		+	K.oxytoca	N	N	N	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	S		R	+		
221	2356	40	M	Urethral stricture	A	P	A	-	-		-	NG																														
222	1246	37	F	Urethral stricture	A	P	A	-	-		+	E.coli	N	N	N	S	S	S	R	R	R	R	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S		S			
223	2123	27	F	CALCULUS	A	P	A	-	-		+	E.coli	N	N	N	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		R	+		
224	2113	49	M	BPH	A	P	A	-	-		-	NG																														
225	2476	79	M	BPH	A	P	A	-	-		+	P.auregnosa	N	N	N	S	R	R	R	R	R	R	R	R	R	S	S	S	R	R	S	S	S	S	S	R	S		R			
226	976	56	M	BPH	A	P	P	+	+		+	Pr.mirabilis	N	N	N	S	S	R	R	R	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	R	S		R			
227	567	68	M	BPH	A	P	A	+	-		+	P.auregnosa	N	M	M	S	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R		S			
228	2876	60	M	BPH	A	P	A	-	-		-	NG																														
229	1956	57	M	BPH	A	P	A	-	-		-	NG																														
230	990	78	M	BPH	A	P	A	-	-		-	NG																														
231	2187	67	M	BPH	A	P	A	-	-		+	P.auregnosa	N	N	N	S	S	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R		S			
232	1678	45	F	Urethral stricture	A	P	A	-	-		+	Pr.mirabilis	N	N	N	S	S	R	R	R	R	R	R	R	S	S	S	S	S	R	S	S	S	S	S	R	S		R			
233	2300	37	F	Urethral stricture	P	P	A	-	-		+	K.oxytoca	M	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	S	S		S	+		
234	1657	89	M	BPH	A	P	A	-	-		-	NG																														
235	1238	67	M	BPH	A	P	A	-	-		-	Pr.mirabilis	N	N	N	S	S	R	R	R	R	S	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
236	875	56	M	BPH	A	P	A	-	-		-	P.auregnosa	N	N	N	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
237	784	45	M	Urethral stricture	A	P	A	-	-		+	CANDIDA																														
238	256	54	M	BPH	A	P	A	-	-		+	K.oxytoca	N	N	N	S	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
239	349	52	M	BPH	A	P	P	-	-		+	P.auregnosa	M	N	M	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
240	429	60	M	BPH	A	P	P	+	-		+	E.coli	M	M	M	S	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		R	+		
241	3561	63	M	BPH	A	P	P	-	+		+	K.pneumoniae	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	S	R	S	S	S	S	S	R	S		S			
242	800	49	M	BPH	A	P	A	-	-		-	NG																														
243	96	50	M	BPH	A	P	A	-	-		-	NG																														
244	905	47	M	CALCULUS	A	P	A	-	-		+	S.aureus	N	N	N	S	S	S	R	R	R	R	R	S	S	S	S	S	S		S	S	S	S	S	R	R	S	R			
245	1176	36	F	Urethral stricture	P	P	A	-	-		+	K.pneumoniae	N	N	N	S	S	R	R	S	R	R	R	S	S	S	S	S	R	S	S	S	S	S	S	R	S		S			
246	1156	60	M	BPH	A	P	P	-	-		+	Pr.vulgaris	N	N	N	S	S	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S	S	S	S	S	R	R		R	+		
247	1185	47	M	BPH	A	P	A	-	-		-	NG																														
248	1187	59	M	BPH	A	P	A	-	-		-	NG																														
249	1167	48	M	BPH	A	P	A	-	-		+	E.coli	M	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	R	S		R			
250	2364	42	M	CALCULUS	A	P	A	-	-		-	NG																														
251	2764	70	M	BPH	A	P	A	+	-		-	NG																														

S.no	OP.NO	Age	Sex	Diagnosis	Fever	Abdomen pain	loin pain	DM	HT	Others	Gram Stain	Culture	Tube Method	Congo Red	Tissue Culture	Cefoxitin	Amikacin	Ampicilin	Gentamycin	Norfloxacin	Ciprofilixacin	Ofloxacin	Cotrimoxazole	Cefotaxine	Ceftriaxone	Ceftazidime	CFCV	CEV	Nitrofurontion	Azteronam	CFS	Imipenam	Meropenam	PTZ	Tetracycline	Cephelexin	AC	Va	CPM	ESBL	AMPC	MR
252	1256	58	M	BPH	A	P	A	-	-		+	P.auregnosa	N	M	N	S	R	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	S		S			
253	2134	50	M	BPH	A	P	A	-	-		+	K.pneumoniae	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	R	S	S	S	S	S	S	R	S		S			
254	1456	54	M	BPH	A	P	A	-	+		-	NG																														
255	1234	57	M	BPH	A	P	P	-	+		+	E.coli	M	M	S	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		S	+		
256	1178	39	F	Urethral stricture	A	P	A	-	-		+	CANDIDA																														
257	1569	56	M	BPH	P	A	A	+	-		+	E.coli	N	M	S	S	S	R	R	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
258	2316	58	M	BPH	A	P	A	+	-		-	K.pneumoniae	N	N	N	S	S	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	R	S		R			
259	271	80	M	BPH	A	P	A	+	-		+	K.pneumoniae	N	N	N	S	S	R	R	R	R	R	R	R	R	R	S	S	R	S	R	S	S	S	S	R	R		S	+		
260	1256	46	M	BPH	P	P	A	-	-		-	NG																														
161	1289	37	F	CALCULUS	A	P	A	-	-		-	NG																														
262	2678	27	F	Urethral stricture	A	P	A	-	-		+	S.aureus	N	M	M	S	R	S	R	R	R	R	R	R	S	S	S	S	R		S	S	S	S	S	R	S	S	S			
263	1145	45	M	BPH	A	P	A	-	-		+	E.coli	N	N	N	R	S	S	S	R	R	S	R	S	S	S	S	S	S	R	S	S	S	S	S	R	R		S		+	
264	256	48	M	BPH	A	P	A	-	-		-	NG																														
265	589	57	M	BPH	P	P	P	-	-		+	K.pneumoniae	M	M	M	S	S	S	R	R	S	S	R	R	R	R	S	S	S	R	S	S	S	S	S	R	S		S	+		
266	2134	68	M	BPH	A	P	A	-	-		-	NG																														
267	1187	60	M	BPH	A	P	P	+	-		+	E.coli	M	M	M	S	S	S	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	S	R		S	+		
268	1167	62	M	BPH	A	P	A	-	-		+	P.auregnosa	N	N	N	S	S	R	R	R	R	R	R	R	R	R	S	S	R	S	S	S	S	S	S	R	S		S			
269	1186	45	M	BPH	A	P	A	-	-		+	Pr.vulgaris	N	N	N	S	S	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R		S			
270	2785	67	M	BPH	A	P	A	-	+		-	NG																														
271	2783	38	F	CALCULUS	A	P	A	-	-		-	NG																														
272	1158	78	M	BPH	P	P	A	-	-		-	NG																														
273	1189	65	M	BPH	A	P	P	-	-		+	E.coli	N	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	R	R		S	+		
274	1146	45	F	Urethral stricture	A	P	A	-	-		+	CANDIDA																														
275	2980	25	F	Urethral stricture	A	P	A	-	+		+	E.coli	N	N	N	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
276	2156	36	M	BPH	A	P	A	-	-		-	NG																														
277	2893	57	F	CARCINOMA CERVIX	P	P	A	-	-		-	K.pneumoniae	M	N	M	S	S	R	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
278	1458	38	M	BPH	A	P	A	-	-		-	NG																														
279	156	57	M	BPH	A	P	A	-	-		+	K.pneumoniae	M	N	M	S	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	R	R	R		S	+		
280	934	84	M	BPH	A	P	A	-	-		-	NG																														
281	2351	57	M	BPH	A	P	P	+	-		-	NG																														
282	3045	36	M	BPH	A	P	A	-	-		+	E.coli	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	R	S	S	S	S	S	S	R	S		S			
283	3048	28	F	Urethral stricture	A	P	A	-	-		-	NG																														
284	1634	73	M	BPH	A	P	A	-	-		-	NG																														
285	1175	46	M	BPH	A	P	A	-	-		+	P.auregnosa	M	N	M	S	S	R	R	R	R	R	R	R	R	R	S	S	R	S	R	S	S	S	S	R	S		S			
286	3089	28	F	Urethral stricture	P	P	A	-	-		-	NG																														
287	1563	69	M	BPH	A	P	A	-	-		+	K.pneumoniae	M	M	S	S	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		R	+		

S.no	OP.NO	Age	Sex	Diagnosis	Fever	Abdomen pain	loin pain	DM	HT	Others	Gram Stain	Culture	Tube Method	Congo Red	Tissue Culture	Cefoxitin	Amikacin	Ampicilin	Gentamycin	Norfloxacin	Ciprofilxacin	Ofloxacin	Cotrimoxazole	Cefotaxine	Ceftriaxone	Ceftazidime	CFCV	CEV	Nitrofurontion	Azteronam	CFS	Imipenam	Meropenam	PTZ	Tetracycline	Cephelexin	AC	Va	CPM	ESBL	AMPC	MR
288	837	58	M	BPH	A	P	A	-	-		+	K.pneumoniae	M	N	M	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
289	694	49	M	BPH	A	P	A	-	-		-	NG																													
290	1563	38	M	Urethral stricture	A	P	A	-	-		+	Pr.mirabilis	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	R	S	S	S	S	S	S	R	S		S			
291	1739	62	M	BPH	A	P	A	+	-		+	E.coli	M	N	M	R	S	R	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		R	+		
292	457	46	M	Urethral stricture	A	P	A	-	-		-	NG																													
293	3123	57	M	BPH	A	P	P	-	-		+	K.pneumoniae	N	N	M	R	S	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	S	S	S	R	R		R		+	
294	1456	37	F	Urethral stricture	A	P	A	-	-		+	CANDIDA																													
295	2863	48	M	BPH	A	P	A	-	-		+	Pr.mirabilis	N	N	N	S	R	R	R	R	R	R	R	S	S	S	S	S	R	S	S	S	S	S	S	R	S		R			
296	1736	51	F	Urethral stricture	A	P	A	+	-		+	P.auregnosa	N	N	N	S	S	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
297	3345	24	F	Urethral stricture	A	P	P	-	-		+	E.coli	N	M	S	S	S	R	R	R	R	R	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S		R			
298	2654	74	M	BPH	A	P	A	-	-		-	NG																													
299	1102	64	M	BPH	A	P	A	-	-		+	K.pneumoniae	M	N	M	S	S	S	R	R	R	R	R	R	R	R	S	S	S	R	S	S	S	S	S	R	R		S	+		
300	2765	54	M	BPH	A	P	A	-	-		+	E.coli	M	N	M	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		S			
301	1654	58	M	BPH	A	P	A	-	-		-	NG																													
302	2609	57	M	BPH	A	P	A	-	-		-	NG																													
303	1673	28	M	Urethral stricture	A	P	A	-	-		+	E.coli	N	N	N	S	S	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S		R			
304	987	35	M	Urethral stricture	A	P	A	-	-		+	E.coli	M	M	M	S	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	R	R	R	R		R	+		
305	2531	48	M	BPH	A	P	A	-	+		-	NG																													
306	1524	56	M	BPH	A	P	A	-	-		+	K.pneumoniae	N	N	N	R	S	R	R	R	R	R	R	R	R	R	S	S	R	R	S	S	S	S	S	S	R		S	+		
307	1273	45	M	Urethral stricture	A	P	A	-	-		-	NG																													
308	1569	56	M	BPH	A	P	A	-	-		+	E.coli	M	N	N	S	S	R	R	R	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	R	S		S			
309	1401	60	M	BPH	A	P	A	-	-		-	NG																													